Title Page

RESISTANCE OF PINE SPECIES AND HYBRIDS TO THE PINE REPRODUCTION WEEVIL

Results of a Cooperative Study

by the

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June 1949

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ABSTRACT

Three years of study (1940 - 1943) of the resistance of pine species and hybrids to insect attack have been completed at the Institute of Forest Genetics. So far this project has been centered on the pine reproduction weevil and on varieties of pine which may be suitable for planting in the brush fields of northern California where the weevil has caused failure of extensive plantings set out 10 to 15 years ago.

A number of species, varieties and hybrids of western pines, (21 altogether) have been tested under controls in the nursery and out-plantings of the Institute of Forest Genetics. Results of these tests have shown wide variation in the degree of resistance to weevil attack between species and hybrids, and between individual trees of the same species. Most of the pine strains that have been tested do not show dependable consistency, either in resistance or susceptibility, with the exception of those named in the following paragraph.

Pinus coulteri is the most resistant of all pine species that have been tested, and cannot be overcome even by forced attacks of heavy weevil populations. One hybrid of Coulter pine, Pinus jeffreyi x (P. jeffreyi x P. coulteri) has also exhibited consistent resistance to strong weevil attacks. At the other extreme, Pinus ponderosa var. scopulorum (Rocky Mountain variety) was found to be highly susceptible to weevil attack and all hybrids of this variety which have been tested exhibit fatally consistent susceptibility.

Test plots of the resistant Jeffrey-Coulter hybrid and of its parent species were set out in the northern California brush fields in the spring of 1948.

Exploratory study of the factors that contribute to resistance and susceptibility of pines have emphasized the need for bio-chemical and pathological studies as an aid to geneticists in further breeding of insect resistant trees.

RESISTANCE OF PINE SPECIES AND HYBRIDS TO THE PINE REPRODUCTION WEEVIL

The testing of strains and hybrids of pines to determine the degree of their susceptibility to certain insect enemies of western pines is a recognized project at the Institute of Forest Genetics. Studies have been carried on during the seasons of 1946, 1947, and 1948 and plans proposed for continuation of the project for an indefinite period. This is a cooperative program of the California Forest and Range Experiment Station of the Forest Service and the Forest Insect Laboratory of the Bureau of Entomology and Plant Quarantine at Berkeley, California.

Up to the present the effects of attack by the pine reproduction weevil, Cylindrocopturus Eatoni, have been studied in some 21 recognized species, strains, and hybrids of pines growing under conditions of the nursery and out-plantings of the Institute of Forest Genetics at Placerville, California. Some species and hybrids have shown much higher susceptibility to weevil attack than others; some have demonstrated a high degree of resistance; and at least one species and one hybrid have demonstrated an inherent capacity to withstand even the most severe attacks of the weevil. (The results of these studies, insofar as results are available up to the present date, will be summarized in this report.) Jorvil

I. REASONS FOR UNDERTAKING STUDY

In the spring of 1946, Dr. Palmer Stockwell proposed that the new hybrids which are being produced at the Institute of Forest Genetics should be studied to determine whether they are more or less susceptible than their parent species to common insect species of pines in the California Region. The reasons for this proposal are obvious. When new strains and hybrids are developed by genetic methods they differ in certain respects from the forms that already exist in nature, and because they are new, we do not know how they will fare when they are exposed to the attack of insects in forest plantations. If there is an inherent weakness to insect attack in a promising hybrid that exhibits desirable silvicultural qualities, it is important to know this before the tree is extensively planted. If the degree of susceptibility is known the geneticist may be able to increase resistance in the strain by further breeding; or if resistance cannot be developed insect control measures must be planned to protect the tree in plantations. These considerations prompted the proposal for specific studies of susceptibility of certain species and hybrids of pine to insect attack, and the facilities of the Institute of Forest Genetics were offered to the Forest Insect Laboratory to institute and further a program along this line.

II. SPECIFIC PROBLEM SELECTED. THE PINE REPRODUCTION WEEVIL.

In planning this project it was agreed that the best chances for progress would be found if preliminary studies were focused on one insect species and on juvenile trees; first, because techniques of procedure and an understanding of the factors involved in tree resistance could best be developed if the study is simplified, and second, because the first years of growth are critical in forest plantings - resistance to insects that attack mature trees loses its importance if the trees cannot survive through the first decade after they are planted.

With the above considerations in mind, selection of an insect species on which to focus preliminary work was largely dictated by the experience of the Forest Service in its planting program in the California Region. One outstanding entomological problem developed in the first large-scale efforts to reforest the brush fields of northern California with native ponderosa and Jeffrey pines. In some areas planted 10 to 15 years ago the pine reproduction weevil has caused severe damage and in one extensive area, the Big Springs Plantation on the Lassen National Forest, large initial investments in preparation of the ground, nursery stock and planting costs, have been completely liquidated by the pine reproduction (Cylindrocopturus eatoni) weevil. Further attempts to reforest these brushfield areas are being held up pending a solution of the weevil problem, either through the development of resistant planting stock which can withstand weevil infestations, or through the perfection of satisfactory and economical control methods.

The best solution seems to be a variety of pine which is adapted to the soil and climate of the brushfields and at the same time is resistant to the weevil. With this as one objective in mind, the initial phases of insect resistance studies were narrowed down to the pine reproduction weevil, Cylindrocopturus Eatoni and to those species, strains and hybrids of pines that appear to be suitable for planting in northern California.

To start with, the prospects for producing a pine variety resistant to the weevil were none too promising. (Although there have been some outstanding accomplishments in plant breeding for resistance to certain diseases, there has been little progress in producing plant strains resistant to specific insects. ~~Plant breeding for resistance to insects contrasts sharply with that in the field dealing with resistance to plant diseases where some outstanding accomplishments have been recorded.~~) Parasitic plant diseases are highly specialized in that the plant cells of the parasite are adapted to feeding on plant cells of the host plant, and apparently a minor change in the nature of the host may disturb this relationship so that the parasite cannot thrive. Insects, however, have much more latitude in the selection of their food. They destroy plant tissues by the process of chewing and digesting them, or by sucking their nourishment from the sap stream. Many species of insects thrive successfully on several species of host plants. In the case of many forest insect species, the tree species that are favorable hosts are so widely separated genetically from non-host species that hybridization to produce resistant strains is impractical. However, in the case of the pine reproduction weevil a cross between one of its favorable host species,

Jeffrey pine, and a non-favorable host, Coulter pine, had been made before this study was initiated. In 1937 a tree that was recognized as a natural hybrid between Coulter and Jeffrey pines was found in the San Jacinto mountains. This hybrid has since been produced artificially. In 1939 pollen from the natural hybrid was placed on Jeffrey pine to produce a back cross in 1949. As will be shown later in this report, the back cross with Jeffrey pine is highly resistant to weevil attack.

III. METHOD USED IN RESISTANCE TESTS Studies

The first tests dealing with weevil resistance were started at the Institute of Forest Genetics in May 1946. The nursery and outplantings of the Institute then contained a wide assortment of pine species, strains and hybrids of suitable ages for weevil attack. In many nursery beds the trees had been left to grow and some fully stocked beds contained trees of ages up to the seventh year of growth. These nursery beds made excellent settings for test cages, especially those in which hybrids were planted in rows with their cone bearing parents, thus making it possible to test both strains under identical conditions.

Testing of these trees for resistance to weevil attack was approached on the purely empirical basis of exposing the trees to weevil populations strong enough to make an effective attack on living trees. To speed up the experiments the expedient of using cages was adopted because there was no assurance when natural field populations of the weevil would become numerous enough for adequate tests. To wait for such populations to develop would leave much of the program to chance

and involve long delays. Another consideration was the necessity for keeping weevil populations in the vicinity of the Institute under control in order to avoid the risk of losing many valuable trees not included in resistance tests. Accordingly trees selected for testing were enclosed in weevil-tight cages, adult weevils were reared under insectary conditions, and these adults were then liberated in sufficient numbers within the cages to insure a lethal attack on all enclosed susceptible trees. (See photos 5 and 6)

The types of cages used are described in the Appendix, pages 13 - 16, and illustrated in the photo section (Photos 16 - 19). These cages were maintained over the trees only during the period of adult weevil attack and early larval development; i.e. from the latter part of May to the first of September. At the close of this period the cages were removed. So far as could be determined enclosure of trees in the cages had no detrimental effect upon growth. In any case a very large part of the seasonal growth had been completed by the time the cages were installed.

Progress of attack in the cages was followed by observation throughout the season. Development of the phases of weevil attack and of injury in the tree was followed by dissections and sectioning of the stem of selected trees at intervals during the season.

In addition to the cage tests natural weevil populations in the area of the Institute provided much additional material for resistance studies, particularly during the seasons of 1946 and 1948.

IV. PROGRESS DURING THE 1946 SEASON

A. Results of cage tests

Three cage tests were carried out during the 1946 season. Each cage housed two parallel rows of trees, one row consisting of a pine hybrid, the other row containing the cone bearing parent of the hybrid. The basic data from these tests and those that were run during subsequent seasons will be found in Table XI of the Appendix. Following is a condensed summary of the 1946 tests. The designation of the experiment is included to serve as a reference to the original notes.

Designation of experiment	Adult weevils in cage	Species and hybrids included	Growing season	Survival
Exp. A	498	8 P. jeffreyi	6th	3 trs. (27%)
		8 P. jeffreyi x (P. J. x P. coulteri)	6th	8 " (100%)
Exp. B	281	P. washoensis	3rd	0 "
		8 P. washoensis x P. ponderosa	3rd	2 " (25%)
Exp. C	90	7 P. ponderosa	3rd	7 " (100%)
		7 P. ponderosa x P. P. scopulorum	3rd	5 " (71%)

In Cage A there was apparently an ample population of adult weevils for effective attack. By mid-summer feeding punctures and resin globules were abundant on the main stems of all trees. There was no apparent discrimination or selection by the weevil between the Jeffrey pines and the hybrids. All hybrids in the cage survived, while 5 out of the 8 Jeffrey pines were killed. While this survival can be expressed in percentage, not much significance can be attached to percentage comparisons because of the limited number of trees in the test.

In Exp. B the weevil population of 231 adults attacked both the Washoe pines and the hybrid indiscriminately and killed all trees in the cage except two of the hybrids.

In Cage C the weevil population of 90 adults was apparently not strong enough for an effective attack on the 14 enclosed trees. Punctures and resin globules were much less numerous than in the other two cages, but two of the ponderosa-occpulorum hybrids were killed.

From a purely empirical analysis the results of these 3 tests indicate that:

1. P. Jeffreyi, P. washoensis, P. washoensis x P. ponderosa, and P. ponderosa x P. ponderosa occpulorum are susceptible to weevil attack.

2. P. jeffreyi x (P. jeffreyi x P. coulteri) and possibly P. ponderosa are resistant to weevil attack.

Deductions from such meager data as provided by these 3 tests were far from convincing. The tests of this season did, however, provide certain leads to follow up, and a considerable amount of experience was gained in techniques of caging trees, rearing weevils, and procedures which could be applied to good advantage in further studies.

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B. Results from attacks by natural weevil populations in the area.

A fairly aggressive weevil population appeared outside of the cages during the 1946 season in the area around the Institute headquarters. Weevils out on the loose attacked and killed 21 P. ponderosa var. scopulorum in the 1923 Progeny Block and arboretum, and 3 P. montana trees in the arboretum. In the nursery these outside populations concentrated in one nursery bed to kill 67 P. murrayana and 3 P. murrayana x P. banksiana in their seventh year of growth. (See Tables III and IV of Appendix). A number of weevils also were attracted to and concentrated around Cage A where they attacked 10 P. jeffreyi x (P. jeffreyi x P. coulteri) all of which resisted the attack. In all of these cases the weevils that attacked outside the cages were able to make a free selection of trees out of a wide assortment of species and hybrids, all within a very limited area. Under such conditions it can be assumed that the trees that were attacked represent a valid preference of the weevil, and indicate the relative susceptibility of pine species and strains. An analysis of the trees that were selected indicate that:

1. P. ponderosa var. scopulorum, P. murrayana, P. montana and P. murrayana x P. banksiana are susceptible to weevil attack.
2. P. jeffreyi x (P. J. x P. coulteri), although attractive to the weevil, is highly resistant.

C. Information obtained from a study of injury in relation to weevil development in susceptible and resistant trees.

The most significant results of the 1946 studies seemed to be the capacity of the Jeffrey-Coulter hybrid to resist fairly strong attacks by the weevil. This raised the question why the hybrid survived when

its cone-bearing parent, Jeffrey pine, was killed under apparently identical conditions for weevil attack.

The first approach to this question was to study the stems of both resistant and susceptible trees to see what went on under the bark after the weevils established their attack. Stems from susceptible Jeffrey pines and from the resistant hybrids that had been attacked in 1946 were examined and sectioned to determine:

(1) If oviposition had been successful

(2) If larval mines had developed

(3) The extent of injury to the inner bark and other tissues of the stem.

In a series of trees that were examined (Table V, Appendix) it was found that the initial phases of the attack, including oviposition and the first larval instar, were similar in both the susceptible Jeffrey pines and the resistant hybrids; but there were marked differences between the two types of trees in the later stages of larval development and in the progress of injury in the inner bark and wood tissues. These differences and their significance are discussed in detail in Section VII of this report, and in pages 17 - 19 of the Appendix.

The overall results of the 1946 study indicated that:

(1) Jeffrey pine and ponderosa pine, both silviculturally desirable species, produce some trees which are susceptible and others which are resistant.

(2) The Jeffrey-Coulter hybrid, which also appears to have excellent silvicultural qualities, is highly resistant since all trees tested resisted heavy attacks by the weevils; and

(3) Other species and hybrids tested exhibit varying degrees of susceptibility.

V. PROGRESS DURING THE 1947 SEASON

It was decided to concentrate part of the study during the 1947 season on the underlying causes of susceptibility in Jeffrey and ponderosa pines to see if the factors involved have any genetic significance; and also to continue resistance tests with as many more species and hybrids of pines as was practicable.

A. Results of cage tests

1. Tests on pure Coulter pines with heavy weevil populations to see if this species is consistently resistant and thus contributes to the resistant strain in the Jeffrey-Coulter hybrid.

Exp. F. Ten vigorous Coulter pines in their third growing season were subjected to attack by 493 adult weevils. All trees were heavily attacked and all survived without serious injury. (Table I, Appendix). omit

2. Further tests on the Jeffrey-Coulter hybrid with heavier weevil populations than in 1946, to see if the resistance shown by this hybrid can be overcome by forces of attack.

Exp. H. Five of these hybrids in their seventh year of growth were subjected to attack by 523 adult weevils. Injury in inner bark, due to necrotic areas, was noticeable but all trees survived. Three out of 4 suppressed Jeffrey pines included in this test were killed. (Table II, Appendix). omit

3. Test on selected Jeffrey pines of high vigor, as nearly as possible equal to that displayed by the Jeffrey-Coulter hybrid, to see if vigor is the dominant factor contributing to resistance.

Exp. G. Three Jeffrey pines in the fourth growing season, with growth rate nearly comparable to Jeffrey-Coulter hybrids, were subjected to attack by 728 adult weevils. One tree was killed and the other two severely injured in the phloem and cambium. (Table 1, Appendix). Growth of the two surviving trees was greatly suppressed in 1948.

4. Tests on pure ponderosa pines as a further check on degree of susceptibility in this species.

Exp. I. Eleven Eldorado strain trees in their fifth year of growth were subjected to attack by 728 adult weevils. Four trees were killed and 7 survived. (Table 1, Appendix).

5. Tests on 5-needle pines to determine whether species of this group are susceptible to weevil attack.

Exp. K. One sugar pine in sixth year of growth was subjected to attack by 510 adult weevils, (Table 1, Appendix). Tree was heavily attacked but survived without serious injury to inner bark.

Exp. J. One Torrey pine in sixth year of growth (grafted on P. ponderosa root stock) was subjected to attack by 486 adult weevils. Stem was heavily attacked both on the Torrey scion and the ponderosa stock, but tree survived (Table 1, Appendix). Injury was sufficient to cause some retardation of growth in 1948.

6. Tests on other available pine hybrids to determine degree of resistance as compared with cone-bearing parents.

Exp. D. P. ponderosa vs. P. ponderosa x P. apacheca. Eight ponderosa pine and 8 hybrids were exposed to attack by 350 adult weevils. All trees survived (Table II).

Exp. E. P. ponderosa vs. P. ponderosa x P. apacheca. Twelve ponderosa pines and 12 hybrids were exposed to attack by 294 adult weevils. All ponderosa pines and 6 of the 12 hybrids survived. (Table II).

Exp. Cal. P. ponderosa vs. P. ponderosa x P. P. scopulorum: Seven ponderosa pines and 7 hybrids were subjected to attack by 583 adult weevils. These were surviving trees of the 1946 test. All trees survived. There is some question of results in this test as the adult weevils used were in a weakened condition due to cold storage. The trees were not heavily attacked. (Table XI).

7. Tests using small cylindrical cages covering only the main stem internodes. The purpose of this was to determine if susceptible trees can be killed when the weevil is limited to certain internodes. If successful this type of cage would greatly reduce the cost of cage materials.

A series of cages enclosing 2 to 3 internodes on 10 ponderosa and Jeffrey pines demonstrated that the weevils would attack the enclosed internodes but no larvae developed. This method of caging was found to be impracticable for further tests. (Page 13, Appendix).

B. Studies of injury in relation to weevil development.

Studies of the development of injury in the inner bark of ponderosa and other pines in relation to the progress of weevil development were carried on more intensively than in 1946. During the period from July 3 to September 24 examinations were made and stem sections prepared from selected trees at intervals of 6 to 8 days. Significant differences were found between susceptible and resistant trees which are shown in Table VIII, Appendix, and are discussed in Section VII of this report.

The more significant points brought out by the 1947 studies seem to stand out about as follows:

1. The Jeffrey-Coulter hybrid shows consistent resistance under caged attack. The force of the attack in 1946 averaged 62 weevils, and in 1947, 104 weevils per tree. The attack of both seasons resulted in injury, but failed to kill any trees.

2. Preliminary tests on Coulter pine indicate that this species is highly resistant and probably contributes to the resistant strain in the Jeffrey-Coulter hybrid.

3. Jeffrey and ponderosa pines continued to show great variability in the resistance of individual trees.

4. Vigor is not the only factor involved in resistance to weevil attack, although it is probably important. It was demonstrated that the weevil can overcome vigorous Jeffrey pines by force of numbers, but more weevils are required than for slower growing trees.

5. Two species of 5-needle pines, sugar pine and Jeffrey pine, were strongly attacked by the weevil but no larval broods developed.

6. The hybrid of ponderosa and Apache pines was less resistant to the weevil than its ponderosa parent.

VI. PROGRESS DURING THE 1948 SEASON

The 1948 cage tests were planned to find out more about the susceptibility of Jeffrey, Coulter, and ponderosa pines, and of the hybrids in which these species are parent strains. Further studies were continued of the differences in reactions of susceptible and resistant pines to weevil attack.

A. Results of Cage Tests

1. Pinus coulteri, to determine whether the resistance of this tree can be overcome by the force of weevil attack.

Exp. I. Six vigorous Coulter pines in the fourth growing season were exposed to attack by 1,369 adults (223 weevils per tree). All trees resisted heavy attacks.

Exp. R. Nine vigorous Coulter pines and 3 suppressed Jeffrey pines were exposed to attack by 1,505 adults (125+ weevils per tree). All 9 Coulter pines resisted heavy attacks; all 3 Jeffrey pines were killed. (Table 1).

2. Pinus jeffreyi, to determine degree of variability in resistance of individual trees.

Exp. P. Fourteen trees in sixth growing season were exposed to attack by 876 adults (62+ weevils per tree). All 14 trees were killed by September 1, indicating very high susceptibility. (Table 1). This result contradicted that of Exp. L, 1947, in which the same trees were tested with 504 weevils (36 per tree) and all survived.

3. Pinus ponderosa, to determine the degree of variability in resistance of individual trees.

Exp. N. Nineteen trees in the sixth growing season were exposed to attack by 816 adults (43 weevils per tree). Six out of 19 trees were killed, a survival of 67%. (Table 1).

4. Pinus ponderosa vs. P. ponderosa x P. P. scopulorum, to determine relative susceptibility.

Exp. G. Six ponderosa pines and 12 hybrids were exposed to attack by 1,190 adults (66 weevils per tree). Four ponderosa pines and 8 hybrids were killed, a survival of 33% in each case. (Table II).

5. Pinus ponderosa vs. P. ponderosa x P. apacheca, to determine relative susceptibility.

Exp. G. Ten ponderosa pines and 8 hybrids in the fourth growing season were exposed to attack by 506 adults (33 weevils per tree). Three out of 10 ponderosa pines were killed (survival 70%) and 5 out of 8 hybrids were killed (survival 37%). (Table II).

6. Pinus ponderosa vs. P. ponderosa x P. arizonica, to determine relative susceptibility.

Exp. E. Five ponderosa pines and 2 hybrids were exposed to attack by 546 adults (78 weevils per tree). Four of the 5 ponderosa pines were killed (survival 20%); both of the 2 hybrids were killed. (Table II).

7. Pinus washoensis vs. P. washoensis x P. ponderosa, to determine relative susceptibility.

Exp. H. Six Washoe pines and 6 hybrids were exposed to attack of 542 adults (45+ weevils per tree). All trees survived (Table II). This result contradicts that obtained from Exp. E, 1946, in which all of the Washoe pines were killed and only 25% of the hybrids survived.

B. Information from Uncaged Species and Hybrids Attacked by the Weevil.

Weevil populations outside of the cages became aggressive during the season and numerous attacks were made, mainly in the nursery beds. This development brought under attack a number of species and hybrids which had not previously been recorded as hosts to the weevil. A list of these species and hybrids will be found in Tables III and IV.

All of these trees killed by the attacks were examined to check on the condition of weevil broods at the end of the season. It was believed that the success of brood development in the stems is a good indication of favorable or unfavorable host species for the weevil.

In the following columns the preferred host species and those that were considered to be non-preferred hosts are listed in the order of success of brood development, the most favorable hosts first, the least favorable last.

Preferred Hosts (Trees attacked, brood development successful)	Non-preferred Hosts (Trees attacked but little or no successful brood development)
1. P. ponderosa scopulorum	1. P. nigra
2. P. ponderosa x P. P. scopulorum	2. P. taeda
3. P. banksiana	3. P. lambertiana
4. P. jeffreyi	4. P. monticola x P. strobus
5. P. ponderosa	5. P. torreyana
6. P. murrayana x P. banksiana	6. P. echinata
7. P. murrayana	7. P. jeffreyi x (P. jeffreyi x P. coulteri)
8. P. sylvestris	8. P. coulteri
9. P. contorta	

No great reliability as to degree of susceptibility can be claimed for the above lists, except for the extremes which have stood out fairly consistently in all cage tests and uncaged attacks by the weevil. Scopulorum pine and hybrids of this strain were highly susceptible; Jeffrey and ponderosa pine were variable; and Coulter pine and the Jeffrey-Coulter hybrid were consistently resistant when attacked.

C. Selection Shown by weevil

A comparison of the preference shown by the weevil between hybrids and their cone-bearing parents was obtained in some of the nursery beds where two strains were planted in parallel rows or randomized so that weevils attracted to the site were given a fairly even choice. Under these conditions a tendency to select one strain over the other could be easily demonstrated. Table IV of the Appendix gives the number of trees attacked and an appraisal of the success of larval development. The following summary compares the number of trees selected in those nursery beds where there was a fairly even choice between two strains.

	Species and Hybrids	Number of Trees Attacked
1.	Pinus ponderosa	14
	vs	
	Pinus ponderosa x P. P. scopulorum	39
2.	P. P. scopulorum	10
	vs	
	P. ponderosa x P. P. scopulorum	5
3.	P. murrayana	67
	vs	
	P. murrayana x P. banksiana	9
4.	P. banksiana	20
	vs	
	P. murrayana x P. banksiana	4
5.	P. nigra	3
	vs	
	P. murrayana x (P. banksiana x nigra)	12

In the first comparison listed above the weevils selected approximately 3 ponderosa-scopulorum hybrids to 1 ponderosa pine; in the second comparison the ratio was 2 scopulorum pines to 1 ponderosa-scopulorum hybrid. Insofar as these results can be accepted as an indication of preferred

hosts of the weevil, they show that scopulorum pine is preferred by the weevil and is more susceptible to the attack than either ponderosa pine or the ponderosa-scopulorum hybrid. The high susceptibility of the hybrid in comparison to ponderosa pine can be attributed to the susceptible strain introduced from its scopulorum parent.

Comparison 3 of lodgepole pine and the lodgepole-jack pine hybrid cannot be accepted as a valid test because of the great preponderance of lodgepole trees in the nursery bed. Lodgepole trees occupied a solid block in which the weevil concentrated; the hybrids were planted in a few rows only at one end of the bed so that the set-up was not comparable to those beds in which the two strains were planted in parallel or randomized in equal numbers. Inaddition to the 3 hybrids that were killed, 17 others were heavily attacked but resisted. Although the conditions for comparison were not satisfactory, this record does show that both lodgepole and the lodgepole-jack pine hybrid are susceptible under certain conditions; also that some degree of resistance is shown by the more vigorous hybrid.

In the fourth comparison the weevils selected jack pine over the lodgepole-jack pine hybrid at a ratio of 5 to 1: and in the fifth comparison the lodgepole-jack pine hybrid was selected over P. nigra at a ratio of 4 to 1. These two comparisons indicate only that P. banksiana is far more susceptible to the weevil than the lodgepole-jack pine hybrid and that P. nigra is not a favored host.

While the evidence presented in Tables III and IV was secured largely by accident in that attacks by uncaged weevils out on the loose could not be foreseen or the tests planned for, enough data was obtained to indicate that susceptibility and resistance to weevil attack is associated with genetic characters which can be transmitted to progeny.

The results of the 1948 tests and studies largely confirmed those of the two preceding seasons. The high lights for this season can be summed up as follows:

1. Pinus coulteri is the most resistant of all pine species which have been tested thus far and was not overcome by forced attacks by heavy weevil populations as is the case with Jeffrey pine.

2. Individual Jeffrey pines which exhibited high resistance in 1947 became highly susceptible in 1948, indicating that changes in the physiological conditions of the tree may be important.

3. Ponderosa pine exhibits varying degrees of resistance and this characteristic was also found in its hybrid with P. P. scopulorum, P. macrocarpa, P. arizonica, and P. washoensis.

4. Uncaged weevils, when free to select the trees for attack, manifested a high preference for P. P. scopulorum (Rocky Mountain form), and for hybrids in which this variety is a parent. P. banksiana also appears to rate high in preference by the weevil. In contrast there is no evidence that the weevil selects P. coulteri in nature.

VII. ~~RESEARCH OF~~ FACTORS INVOLVED IN THE SUSCEPTIBILITY OF PINES TO WEEVIL ATTACK

In the preceding account of ~~these~~ experiments and studies we have described only the easily visible effects of weevil attack upon various species and hybrids of pines. Whenever the trees ~~were~~ exposed to strong weevil populations under cage control, all species and hybrids tested were attacked. Some trees were killed by developments following the attack and some survived. Some species and hybrids ~~appeared to be~~ were obviously more susceptible than others, but even among the more susceptible strains some individual trees ~~exhibited a capacity to~~ resisted the attack and survived.

Why were some trees killed and why were others able to successfully resist the attack? This question becomes highly pertinent from the genetics viewpoint in those cases where trees of the same age, growing under identical conditions, were attacked with equal intensity by the weevil. The evidences of attack in the outer bark (punctures, resin flow, and necrotic areas) were similar in all trees. But in susceptible trees, larvae developed rapidly, extended their mines through the inner bark, and by the time that these mines had entered the sap wood the tree was either dying or dead. In the resistant trees the larvae were contained in the cortex where they eventually died, and their mines failed to penetrate the deeper phloem and cambium layers.

Superficially then it is fairly obvious that the capacity of pines to resist weevil attack can be narrowed down to the physiological characteristics of those layers of plant tissue between the outer bark and the sapwood. In resistant trees it may be some inherited chemical or physical properties of the inner bark that makes this part of the tree untenable for weevil larvae; this most certainly must be true for those species of pines and other conifers which the weevil does not attack and kill in nature. Susceptibility on the other hand may be the result of changes in the physiological condition of the inner bark, resulting from unfavorable environmental conditions for the tree or other causes which break down inherent resistance. In any case, the underlying causes of susceptibility to the weevil in pines cannot be found until we know more of the processes that go on within the inner bark when this part of the tree is invaded by weevil larvae.

In 1947 an effort was made to find out just what happened to the larvae in the outer and inner bark of both susceptible and resistant trees, and to see if any conditions could be recognized in the cortex, phloem, cambium and outer sapwood which would help to explain the success or failure of weevil attack.

The information that was obtained can best be presented by a review of the progressive stages of development in the seasonal history of the weevil. As found by Eaton* and verified by all observations in the

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* Eaton, C. B. Biology of the weevil, Cylindrocopturus eatoni, Buchanan. Injurious to ponderosa and Jeffrey pine reproduction. Journal of Economic Entomology 35/, Feb. 1942.

Placerville studies, the pine reproduction weevil attacks only living trees. Its general life cycle in these trees which it succeeds in killing is illustrated in Figure 5, page 37.

Punctures on the needles. The newly emerged adults first attack the pines to which they are attracted by making punctures through the epidermal tissue of the needles. This is considered to be a feeding habit necessary for the full maturity of the adult. No great injury to the tree seems to result from the punctures, even when they are numerous, and the habit has been dismissed as an essential phase of the processes that bring about the death of the tree. However, an experiment with small cages, in which the adult weevils were enclosed only with the main stem of the internodes, (the nodes, limbs and needles being excluded, as described in pages 16 and 17 of the Appendix) indicate that needle punctures may be of more significance than was at first supposed. Pine needles from other trees were kept in the small cages, in order that the adults would have access to their normal food material. However, ponderosa pines were killed by this restricted type of forced attack only when weevils outside the cages attacked the unaged nodes and branches. These results suggest the possibility that some virus may be associated with the needle punctures which first conditions the tree for the attack that follows on the main stem. Further study along this line can probably be followed up with profit.

Punctures on the stems. From the needles the adults go to the bark of the main stem and branches where they make similar punctures to those on the needles. Usually these attacks on the stems are concentrated on nodes and internodes from 2 to 5 years of age. Stem punctures are often numerous, as many as 50 to 100 having been counted per linear inch of stem. Whenever a puncture severs a resin storage canal in the cortex, resin pours out to the surface and forms a bonillike globule. (Photos 6, 7, and 8). The number of these resin globules and the volume of resin produced varies greatly in individual trees. In ponderosa and Jeffrey pines the amount of surface resin produced does not seem to be related entirely to vigor — some suppressed, slow-growing trees producing more resin than fast-growing trees. Coulter pines always produced a conspicuous amount of surface resin globules. So far it has not been demonstrated that the quantity of surface resin produced is related to successful resistance to weevil attack, although there is some indication that this may be the case.

Necrotic areas. Soon after the punctures are made, the cells bordering the cavity die, become impregnated with resin, and turn to a dark brown color that contrasts sharply with the green living cells of the outer cortex. At first only cells adjacent to the punctures discolor, indicating that they died from mechanical injury caused by the chewing activities of the weevil. But the deadened area around the punctures may soon extend into more of the living cortex within 2 or 3 weeks after the first attack many of the punctures are surrounded by large

patches of dead resinous tissue, which are termed necrotic areas, (see photos, 9, 10, 24 and 25). Most of the necrotic areas are considerably larger than would be expected from the effects of mechanical injury bordering the puncture.

The area and depth of individual necrotic areas vary greatly in the same tree and in different trees (page 7, Appendix). In many respects necrotic areas caused by the weevil are quite similar to those that surround punctures in living pine bark caused by the Prescott scale:* With both the weevil and the scale the nature of the deadened tissue around the punctures is analagous; in neither case does mechanical injury seem to explain the extent of the deadened tissues; in both cases healthy trees soon wall off the necrotic area with a thin layer of cork cells; but in neither case has a specific organism been found which will account for the spread of necrotic tissue around the initial punctures.

In the case of the weevil, necrosis is confined to the cortex; rarely if ever do the necrotic areas extend deep enough to affect the phloem and cambium. Necrotic areas are similar in both susceptible and resistant pines, and so far as has been determined in these studies, they have very little to do with the ultimate death of susceptible trees. As will be shown later, however, necrotic tissue does seem to be highly important to the first stage of larval development of the weevil.

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* McKenzie, Gill and Ellis. The Prescott Scale (Matsucoccus vexillorum) and associated organisms that cause flagging injury to ponderosa pine in the Southwest. Jour. Agr. Research, Vol. 76, No. 2, Jan. 15, 1948.

Oviposition of the weevil. A small percent of the punctures made by adult weevils on the stem serve as receptacles for the eggs. In these studies eggs were not found until the necrotic areas were well formed. The eggs that were found had been placed in pits alongside of well developed necrotic areas, but none were found in the resinous necrotic tissue.

First stage larvae. Soon after the eggs incubate the first instar larvae begin feeding along the borders of or in the necrotic areas. The first feeding mines found were always very closely connected with necrotic tissue, indicating that this provides some essential nutritional requirement of the first stages of larval growth.

Later larval development. Up to and including the first stages of larval growth, developments, (both of the insect and of tree responses,) were apparently identical in both susceptible and resistant trees. But from this point on larval development in susceptible trees followed a pattern distinctly different from that in the resistant trees. For this reason it will now be necessary to differentiate between these two types of trees.

A. In susceptible trees.

Practically all susceptible trees formed a distinct layer of cork cells to wall off the necrotic areas in the cortex, although this was fairly faint in some cases.

Mines were taken to be first instar larvae were first found in or alongside of necrotic areas in late July and early August; during the last half of August distinct larval mines were being extended into the living cortex and phloem; by the first of September many mines had

penetrated to the sapwood (Photo 26); and during the first week in September larval mines, which penetrated the phloem and cambium layers and scored the surface of the sapwood, were found in all susceptible trees. When development had reached this point the tree was either dying or dead. During September and October the larvae completed their feeding and then burrowed into the wood or pith where they went into the overwintering condition in the full grown stage (Photo 27). Typical of this development in susceptible trees are trees No. I-7, I-8, I-10, I-11, and I-13, listed in Table VIII.

B. In resistant trees

All resistant trees rapidly walled off the necrotic areas with a layer of cork cells so that the process of necrosis was contained within the cortex. So far as could be determined, this process did not differ from that in susceptible trees.

During all of August and through the entire fall and winter, living first stage larvae were found only in or alongside of the necrotic areas. A larval mine that was contained entirely within the necrotic area is shown in Photo 14. There was evidence that some of these larvae had attempted to extend mines away from the necrotic tissue and into the green cortex and phloem, but all died, usually in short mines that were heavily infiltrated with resins. Many first stage larvae remained alive in the necrotic areas during all of the fall and winter, but made no appreciable growth beyond the first instar. All surviving

larvae died, however, in the spring about the time that the tree began its seasonal growth. Trees No. 1-9 and 1-12, Table VIII, and trees X-1 to X-10, Table V, Appendix, are typical of development in resistant trees. In some of these infected trees which were cut off at the root collar for study in October and November, (and were kept in the laboratory,) the retarded first stage larvae started development and reached the full grown stage in 4 to 6 weeks. In trees which were similarly treated in February and March following the overwintering period, these retarded larvae failed to develop further and soon died.

To summarize this comparison between susceptible and resistant trees, the overall differences noted are:

1-a. In susceptible trees the larvae, after leaving the necrotic areas, developed rapidly in the living cortex, entered the phloem and cambium layers, completed their growth and the trees died before the end of the season.

2-b. In resistant trees the larvae were unable to develop in the living cortex, their growth was inhibited throughout the entire season, and they eventually died in or near the necrotic areas. These resistant trees were able to contain the attack within the outer cortex and thus survived.

FACTORS CONTRIBUTING TO SUSCEPTIBILITY IN PINES

The data that has been obtained as well as the cross sections of trees illustrating developments in the bark and sapwood present only an incomplete post mortem picture of what occurred in pines during progress of the weevil attack. The question why some trees were able to contain

the attack in the outer bark while others were unable to do so still remains unanswered. Although no answer is possible from the data obtained, some factors can be considered which seem to point, inferentially at least, to the causes of susceptibility.

One approach to the question is to assume that all pines have some inherent capacity to resist insect attack, and that this character is weakened by adverse factors. Among these we can consider:

1. Conditioning of the tree for attack by some disease or virus. No direct evidence was obtained to indicate that susceptible trees are weakened by an organism of this character.

2. Necrosis of tissues in the bark around weevil punctures. Both susceptible and resistant trees are capable of arresting necrosis and necrotic areas seem to have no significance unless they may be necessary to larval nutrition.

3. Character of resin production
   A - In the cortex.
   No differences between resistant and susceptible pines could be found in either the character or quantity of resin ducts in the areas. In both types of trees resins flowed to the surface when the resin vessels were ruptured by the attack. However, in resistant trees resins infiltrated the first larval mines and apparently caused death of the larvae.
   B - In the sapwood.
   Differences between resistant and susceptible trees were found in the production of resin canals in the outer sapwood following the initial phases of weevil attack. In resistant trees a heavy concentration of resin ducts developed in the outer sapwood, which was apparently stimulated by weevil attack. This concentration was much heavier in some trees than in others. (See Photos 25, 28, 29, and 30). All trees that were cross-sectioned showed some measure of stimulated resin production. This tendency was striking in Coulter pines, as well as in some resistant ponderosa pines.
   In susceptible trees there was great variation in the quantity resin ducts produced after attack. In some trees (1-10, 1-9, and 1-12, Table VIII) production of

resin ducts following the attack was entirely lacking (see Photo 26). In other trees these ducts were present, although their number and size were limited in comparison with those found in resistant trees.

4. Soil moisture deficiency.
In the nursery the trees were irrigated at intervals during the 1946 and 1947 seasons, with the overhead sprinkler system. In 1948 irrigation was discontinued in all of the beds in which weevil tests were being carried on. In comparing the volume of weevil attack outside the cages during these 3 seasons, we find that many more trees were attacked in 1948 than in 1947. One striking contrast is found in Experiments P and L (Table 1, Appendix). In 1947, 14 Jeffrey pines with periodic irrigation, proved to be 100% resistant: in 1948, the same 14 trees without irrigation, proved to be 100% susceptible. An unknown factor in this test was the relative virility of the adult weevils that were liberated in the two cages; there is some question that the adults liberated in 1947 were in good condition because of their having been kept for some time in cold storage. Also, 374 more weevils were liberated in 1948 than in 1947, but the population liberated in 1947 should have been ample to kill the trees if they had been susceptible. That there was a change in susceptibility in 1948 is indicated by the fact that a large number of trees outside of the cage were attacked and killed in the same nursery bed, as compared with none in 1947.

Apparently there is some evidence to support the theory that a deficiency of soil moisture during the period of weevil attack induces susceptibility in certain species of pines, which would otherwise be resistant.

Other factors that may be considered are the bio-chemical conditions in the inner bark, especially those that pertain to the sugars and starches necessary to nutrition of weevil larvae.

In general, what evidence we have points to the nature and quantity of resins produced under the stimulus of weevil attack as an important factor in the resistance of pines; also to soil moisture deficiencies as a factor contributing to susceptibility of pines. No conclusions seem warranted, however, until much further work has been done on these phases of the problem.

VI Further Studies
1. Field plantings

~~VIII. FIELD PLANTING TESTS OF WEEVIL RESISTANT PINES~~

After it was found by the simple method of cage tests that the hybrid P. jeffreyi x (P. jeffreyi x P. coulteri) was more resistant to the weevil than Jeffrey and ponderosa pines, the proposal was made to test this hybrid in the brushfield planting areas where Jeffrey and ponderosa had failed. The young hybrid trees growing in the Placerville nursery exhibited some promising silvicultural characteristics which made them desirable for planting purposes in the northern California areas. In growth vigor it was superior to Jeffrey pine, and at the same time it can be expected to possess some of the fine wood qualities of this parent. In the spring of 1948 plans were made to set out test plots of the hybrid, along with Jeffrey, Coulter and ponderosa pines as checks. Three plots, each containing 100 trees of both the hybrid and Jeffrey pines with 20 Coulter pines, were established under a cooperative arrangement of the Forest Service, the Institute of Forest Genetics, and the Berkeley Forest Insect Laboratory. A description of this project can be found in a report by R. C. Hall. Results from this test will not become available for several years.

Hall, Ralph C. Tests of hybrid pines for resistance to the reproduction weevil under field conditions. July 15, 1948.

A- (work page 33- last para)

IX. SUGGESTED PROGRAM FOR FURTHER STUDIES OF INSECT RESISTANCE IN PINES

For the past three seasons investigations of weevil resistance in pines have been limited to the material and facilities available at the Institute of Forest Genetics. The conditions afforded for the project have been excellent, but the work has been carried along mainly on the basis of expediency since no funds or authority for a continuing project have been available. The approach to the study thus far has been mainly exploratory, with the thought in mind that long range planning could be done to the best advantage after a few years of experience had pointed out the most promising leads to follow. The project now seems to have reached the point where, if the study is to continue, it should be pointed up as a long term program with specific plans and objectives. The following suggestions are intended to serve as a guide to further developments.

A. Special test nursery for resistance and selection studies

In looking ahead to continuation of resistance tests at the Institute of Forest Genetics, the need for a small nursery devoted entirely to this purpose is obvious. This was realized soon after the studies were started at the Institute and in the spring of 1946 a small nursery was set out to serve as material for resistance testing. Details and purposes of this planting are covered in a report by the writer dated July 15, 1948. The plan calls for complete housing of two nursery beds in

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Miller, John M. Plans for further insect resistance tests at the Institute of Forest Genetics. July 15, 1948.

one insectary-type cage to confine the weevil population with a large number of species and varieties of pines. The purposes to be served by the test nursery are:

1. Isolation of the weevil testing program from the regular nursery and arboretum of the Institute.
2. Greater variety and better arrangement of the trees for the tests.
3. Selection by the weevil of its preferred hosts from a large number of species and varieties of pines (The limitations of the small cages that have been used up to the present time are discussed in the above report).

B. Extension of field plantings

The ultimate test for weevil resistance will come when the trees are set out in the field plantings. A start along this line was made in 1948 by the plots in brushfield areas described above. These provide, however, for a preliminary test of only one hybrid.

Proposals are now being considered for a 2- to 3-acre planting which will be large enough to accommodate all of the species and hybrids which may be suitable silviculturally for northern California brushfield areas. When the trees reach suitable age weevil populations will be maintained in the area so that all trees will be exposed to attack under natural conditions.

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C. Tree breeding to improve resistance in desirable strains of pines

This must be a continuing program carried on by geneticists. To a large extent progress must wait on the development of studies to determine the relative resistance and susceptibility of species and strains which can be used to produce crosses or can be selected for vegetative propagation. A start has already been made in this field by the Jeffrey-Coulter hybrid in which introduction of the resistant Coulter strain has apparently resulted in a resistant F_2 hybrid. However, the results of breeding experiments for weevil resistance can be prosecuted to better advantage when more is known about the underlying causes of susceptibility.

D. Studies of factors that contribute to the resistance or susceptibility of pines

1. Bio-chemical studies to determine the nature of the resins that are found in the inner and outer bark, their toxicity to different stages of development of the insect, and the differences in resin production between resistant and susceptible trees.

2. Pathological studies to determine whether any organisms are involved in weakening the tree so that it becomes susceptible to attack and provides conditions favorable to nutrition and development of weevil larvae. Necrotic areas suggest that some organism is associated with the weevil in its invasion of the inner bark of the tree.

3. Investigation of the edaphic factors, including soil type and soil moisture, which may be unfavorable to the tree and thus contribute to its susceptibility.

APPENDIX

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APPENDIX

I. SEASONAL HISTORY OF CYLINDROCOPTURUS FURNII

In the studies at Placerville no important differences were found from the life history cycle worked out by Eaton in northern California. Emergence dates and the period of adult activity varied some from Eaton's records, but these were no greater than the differences found between different years in the Placerville area.

1. Sources of Rearing Material.

To secure adult weevils for stocking the test cages, infested trees containing overwintering broods of mature larvae were collected in the spring and held in rearing cages or cans until all the broods had transformed to new adults and emerged. The new adults were trapped in glass jars as they emerged and were then liberated in the test cages as soon as practicable. By this procedure it was possible to count the exact number of adults liberated in each cage. Infested material from the following sources was used:

1946 Material. Twenty infested ponderosa pines, 5 to 10 years of age, were collected at Cloppe Summit, Sierra National Forest, in early April 1946. They were stored in the basement of the Hasel Field Lab until May 31, when they were taken in to Berkeley. At that time the overwintering brood was found to consist of pupae and new adults. To hold back emergence until the field cages were ready, the trees were taken to Berkeley where they were held in a 40 degree F. cold storage room until June 10. They were then taken to Placerville and placed in rearing cages. About 900 adults were reared from this material.

1947 Material. An abundance of infested material was found for this year's tests in the tops of about twenty 14-year-old scopulorum pines at the Institute. This material was allowed to overwinter in the open and was placed in the rearing cans in early April 1947, when the first pupae began to appear. About 11,500 adults were reared this season.

1948 Material. Two infested scopulorum pines, 15 years old, in the arboretum, provided most of the adults for the 1948 tests. In addition, 20 infested Jeffrey pines from the Burney Brush Field Plantation on the Lassen Forest were brought in to supplement the emergence from the scopulorum pines. All of these trees overwintered in the field and no pupae had yet formed when they were put in rearing cans about May 10. About 7,600 adults were reared from all this material.

2. Notes on Rearing Difficulties

Rearing cages were kept in the seed laboratory of the Institute, where temperatures were fairly close to those of the outside air. As the adults emerged considerable difficulty was encountered in keeping them from escaping from the rearing cans and emergence jars. On hot days they were extremely active and seemed able to find their way out through the smallest of openings and seams in the containers. Galvanized iron cylinder-shaped rearing cans proved to be the best type of cage to prevent this leakage, but moisture conditions within the cans were difficult to regulate and caused some trouble because of moulds. In spite of soldered joints and tight fitting metal lids on these cans, some adults did escape and found their way to the windows of the seed lab. These escaped adults were disposed of by spraying the windows with DDT emulsion.

During emergence peaks adults came out faster than they could be accommodated in the test cages. To keep them quiet until they could be liberated they were held in a refrigerator at 36 - 38° F. This treatment seemed to have no detrimental effect for the first few days or a week as all adults revived and began normal activity as soon as they warmed up. It was noted, however, that prolonged exposures to cold lasting for 10 days or 2 weeks produced shock from which adults were very slow to recover. Many of them that had been held in the refrigerator for this length of time did not recover at all but died in the container jars.

3. Emergence Periods of New Adults from Rearing Cages.

Wide seasonal differences were found in emergence periods as shown by the following summary:

Season	First Emergence	Peak	Last Emergence
1946	June 1 - 3*	June 13	June 29
1947	April 26 - May 1	May 24 and June 9	June 30
1948	June 14 - 25	July 4	July 16

Emergence curves for each season are graphed in Figure 1. The seasonal differences are in part accounted for by preliminary handling of the material. The 1946 record was delayed because of cold storage, but the 1947 and 1948 records are very close to emergence as it would have occurred under natural field conditions. The difference in first emergence between these two seasons was more than 40 days, 1947 having an early spring with very warm weather in May; 1948 had a cold, late spring and almost no warm weather even in June. In 1947 there were two pronounced peaks of emergence, corresponding to two short spells of hot weather, one on May 24, the other on June 9, separated by a relatively cold spell.

4. Activity of the Parent Adults.

A. Feeding on the needles. This was observed in all cages within a day or two after the weevils had access to the trees. The habit as observed in the test cages was essentially the same as that described by Eaton.

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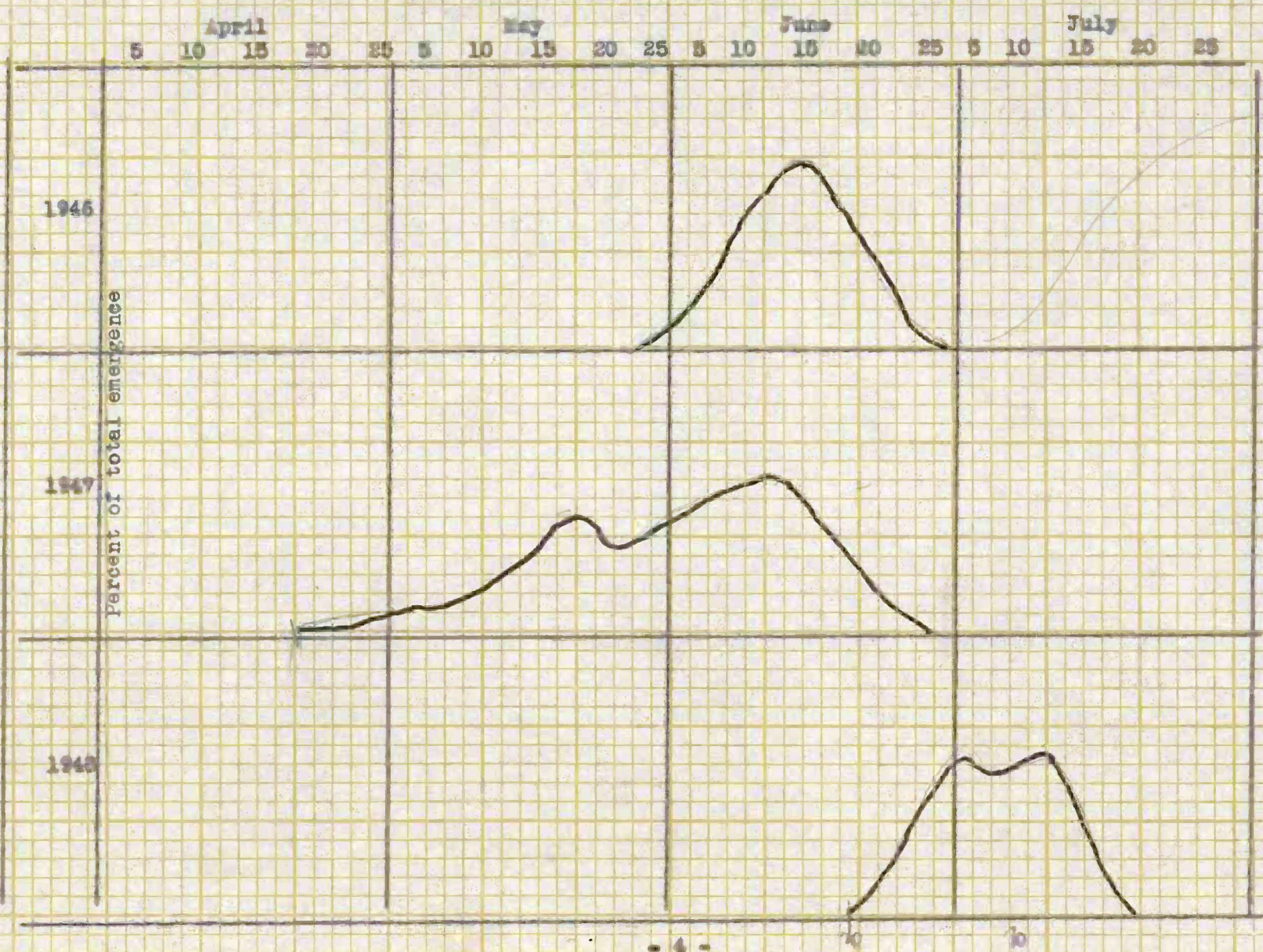
* First emergence was held back by storage in refrigerator. Normal emergence for this season would probably have been about May 10 - 20.

Figure 1.- Emergence of new adults of Cylindrocopturus eatoni from rearing cans at Institute of Forest Genetics.



B. Feeding and Oviposition Punctures on the Main Stem. Adults were observed feeding on thin bark of the main stem within a week or two after they were liberated in the cages. This activity continued through July and August, although the number of adults that could be seen feeding on the stems decreased as the season advanced. In 1947 this activity was followed closely on a series of selected ponderosa pines in one cage. The greatest number of active weevils were observed during the first three weeks after they were liberated, as the season advanced during July and August the weevils noted on the stems became less active, they stuck closely to the bark, and were difficult to find. By the end of August the adults could scarcely be dislodged even by vigorous shaking of the tree. The last adults were found on stems on September 19 — after that date none were found. One characteristic of this habit of feeding and oviposition on the stems was the tendency for large numbers of the adults to concentrate on certain trees. As many as 25 weevils were found on the stem of one small tree in late August, while only two or three were found on other trees in the same cage.

C. Reaction to Light. Adults emerging in the rearing cages did not seem to be strongly phototropic. Practically all weevils eventually found their way into the lighted emergence jars, but, as noted by Eaton, there was a tendency on the part of some weevils to remain for some time in darkened parts of the cages. However, after emergence the adults seemed to prefer trees for attack that were located in sunny exposures, and to avoid trees that were in heavily shaded positions. Small suppressed trees in the lower part of the cages were lightly attacked, if at all, unless they received good exposure to the sun during part of the day. This tendency to select trees in good sunny exposures was best demonstrated in the nursery beds by weevil populations outside of the cages. In heavily stocked beds of 5- and 6-year-old ponderosa and Jeffrey pines, fairly uniform in size and vigor, most of the trees selected for attack were along the south side of the beds where a considerable amount of sunlight reached the lower internodes.

D. Resin Flow in Stem Punctures. Resin globules began to appear on the stems of caged trees within a week or 10 days after the weevils were introduced into the cages. This resin flow came from resin storage canals in the cortex and phloem that were severed when the weevil made its initial puncture, but all punctures did not produce resin. Close examination of the stems showed that many punctures were "dry holes" producing no resin flow whatever. On one tree (1-5, ponderosa pine) a count showed that there were 100 of these small punctures, only one-quarter of which produced resin flow, on one linear inch of stem where the attack was concentrated on the 1943 internode. This was considerably heavier than average, the more heavily attacked probably averaging from 25 to 50 punctures per linear inch of stem. There was great variation, however, in the number of punctures on different internodes of the same tree, the heaviest concentrations occurring usually on the 2nd and 3rd internodes below the current season's growth. The amount of resin produced varied greatly in different trees, but all attacked trees produced at least some resin globules on the bark surface.

E. Necrotic Areas. Around each puncture an area of desiccated, resin-filled cells, termed "necrotic areas," formed in the cortex tissues within 10 days to 2 weeks after the attack. These areas varied greatly in size. Some remained only as small caps of brown tissue surrounding the punctures; others progressively developed into large, irregular areas, indicating that something more than the mere mechanical injury of the puncture was responsible for deadening of the tissues. In all living resistant trees these necrotic areas were contained in the outer cortex by a layer of cork cells which developed under them; some spread deep into the cortex tissues but none penetrated the phloem and cambium layers unless the tree died.

5. Eggs.

Eggs of the weevil, as explained by Eaton, are very difficult to find because they are very small and more or less covered up by resins. A careful search was made for the eggs only during the 1947 season. During the period from July 1 to September 25 of this season development of eggs and larvae were followed in a series of caged ponderosa pines. The first eggs were found July 15 (Table VIII); these were in pits in the living cortex, but were always found along side of necrotic areas — a few seemed to be placed in the necrotic areas. By July 31 eggs were numerous and continued to be present in the cortex during August. The last eggs were found in a tree examined September 5. From this it would appear that oviposition goes on through most of the active season, although the bulk of the larval broods come from eggs deposited during July and early August. Development from eggs laid in September, when most of the larval broods in susceptible trees are approaching the full-grown stage, could not be closely followed up, but it is probable that, given a warm fall, eggs deposited this late may reach the full-grown larvae stage by November.

6. Larvae.

The first larvae were found in the outer cortex of a tree that was examined July 31 (See Table VIII). These were in frass-filled pits along side of or in the necrotic areas. A large number of eggs were found in this same tree. There was fairly good evidence the first feeding of the newly hatched larvae takes place in the necrotic areas. These first stage larvae are very hard to find because of the resinous material in which they are imbedded.

In a tree examined August 6 larvae were fairly abundant. These were still quite small, but a number of them were extending mines into the living cortex tissues away from the necrotic areas. The same stages of larvae were found in the trees examined August 14 and August 27. In the tree cut September 5 the larvae found were in varying stages of development; many were still in the outer cortex but some had penetrated through the phloem and cambium and had reached the sap wood. This tree obviously was not resisting the attack and was in the process of dying. In the tree examined September 19 only small first stage larvae were found in or alongside of necbotic areas. This tree was apparently resisting the attack. These differences in stage of larval development in different trees are apparently due to resistance in some trees which inhibits normal growth of the larvae, rather than to differences in the timing of the attack.

7. Pupae and New Adults Found in September and October.

In his studies at Hat Creek, Eaton found a few pupae and new adults in pupal cells during late September, in trees that had been killed that same season. He did not, however, observe emergence of these new adults that fall. At Placerville the development of a few new adults in the fall from larvae of the current season was observed in 1946. Following are the note records:

> September 6, 1946. In examination of small Jeffrey pine killed during August found several pupae in pith of main stem. On September 9 found one new adult in pupal cell of same tree.
>
> September 12, 1946. Examination of a Washoe pine killed during summer revealed several pupae and new adults in pupal cells. This tree and the infested stem of a scopulorum pine were put in rearing cage.

September 18 to 30, 1946. Twenty-three adults emerged, along with some parasites. The adults from this emergence were liberated in a small cage enclosing a small potted P. ponderosa pine in the fourth growing season, to see if adults emerging in the fall would attack living trees. On October 1, observed 1 adult in the cage feeding on needles and several resin globules on the twig stems of the caged tree. The weevils all died off by November 1 without establishing a successful attack on the tree.

In these observations it was noted that pupae and new adults were found in the fall only in small trees which had died before September 6, and that the stems had become very dry. No checks on fall pupation and emergence were made for the seasons of 1947 and 1948. From Eaton's observations and from the Placerville records it is obvious that this habit of pupation and emergence of new adults in the fall involves only a small percent of the total seasonal population and that no attacks of any consequence occur on living trees.

8. Overwintering Stages of the Larvae.

All observations confirm Eaton's conclusion that the great bulk of the weevil populations overwinter in the larval stage. But in considering the data obtained in the Placerville studies, it is necessary to break down development into two categories because marked differences in the development of overwintering larvae were found between susceptible and resistant pines.

A. In Trees Killed by Seasonal Attack of Weevil. Most of the trees that failed to resist the attack were dead by October 1. There was, however, some variation according to season and some trees remained green during the fall and did not turn red until sometime in the winter or early spring. In those trees that failed in the fall the main brood of larvae had reached the full grown stage by October and had burrowed into the wood to form their overwintering mines. In trees that did not fade until later the larval broods reached the full grown larval stage by the time winter arrived. The term "full grown" as used here applies to those larvae that excavate a mine into the wood in which they remain dormant during the winter. (A very few of these mines do not penetrate far into the wood and the overwintering cell is found just under the bark). It can be concluded that, in susceptible trees, the infestation overwinters in the full grown larval stage in the wood or pith. There is a long dormant period lasting from about October 15 to April 15, and pupation occurs in April and May, depending upon the season.

B. In Trees Which Resisted the Seasonal Attack. Resistant pines were found both inside and outside of the cages during the three seasons that studies were carried on at the Institute of Forest Genetics. That many of these resistant trees had been heavily attacked by adult weevils during the summer was indicated by pitch exudations on the stem and branches and by well formed necrotic areas that could be found under the bark. It was assumed that such trees were resistant to weevil attack if they remained green through the winter and resumed growth the following spring.

During the fall of 1946 and the ensuing winter a number of resistant trees were examined at intervals to determine whether any larvae developed in the cortex areas. In practically all such trees tiny first stage larvae were found throughout the fall and winter; some were in necrotic areas, some were attempting to mine the living cortex, and some were dead.

It was characteristic of all larvae found in resistant trees that no perceptible growth had been made beyond the first stage of instar. Some living larvae were found until late March, but after April 1st all larvae found were dead. It was noted that this complete mortality was not found until the trees had started new growth for the season. In Table V is a list of observations in 7 trees that were followed during the fall, winter, and spring of 1946 - 47. In all 7 trees only first stage larvae were found when the trees were first examined and ~~that~~ no perceptible growth beyond this stage was found in later examinations. After March 20 most of the larvae found were dead, either in the necrotic areas or in short, resin-filled mines in the cortex. Other examinations of resistant trees that were attacked during the 1947 and 1948 seasons confirmed the conclusion that resistant trees may contain living, first stage larvae through the fall and winter, that these larvae are inhibited and do not grow, and that they eventually die by the time that the tree begins new growth in the spring.

C. In Resistant Trees Cut During the Fall. That these inhibited larvae are capable of growth and development if the tree changes from a resistant to a susceptible condition was discovered in the fall of 1946. It was found that when resistant trees are cut off at the ground level and kept at temperatures above 60° F., the inhibited larvae start growing, develop very rapidly and mature the same as larvae in trees killed by the weevil. This point was discovered incidentally to the examinations of resistant trees listed in Table V. In the fall of 1948 this point was checked further for several species by cutting a series of resistant trees and holding them under temperatures that would permit larval development. Table VI presents the records from resistant Jeffrey, Coulter and ponderosa pines cut for this study. The first stage larvae started growth and matured normally in all species except Coulter pine. An earlier test for which the trees were cut on September 4 showed no larval development in any trees, but it was evident that these trees had dried out very rapidly during a hot spell of weather during September and all of the young larvae died as a result.

II. NOTES ON CAGES USED

The main purpose of the cages used in these tests was to enclose selected trees in a type of cage which would not interfere with normal growth of the trees, and at the same time hold adult weevil populations in sufficient numbers to insure an effective attack on enclosed trees. The weevils were thus forced to attack the trees inside the cages. Once the attack was established and the larval broods were developing under the bark, the cages served no further purpose in the tests and were removed.

4. Type of screen used. The cages used to cover trees were constructed with a wood frame work supporting a screen fine enough to prevent the adults from coming through the mesh. In preliminary tests it was found that the smaller weevils could work their way through the screen if it was coarser than 24 mesh to the inch. When the first cages were constructed in the spring of 1946 24 mesh screen could not be obtained on the market and the only available material was salvaged screen from some old cages of the Forest Insect Laboratory. There was not enough of this to cover the three cages needed that season, so the supply of screen was supplemented by using muslin on the side walls on the larger cage (Photo 17). This held the weevils but induced high temperatures within the cage. Smaller cages with screen side walls and top (Photo 16) were fairly satisfactory.

A number of cages were needed for the season of 1947. Twenty-four mesh screen was still not available, so surgical gauze with a 24 x 20 mesh was substituted. This material held the weevils fairly well and was inexpensive; its disadvantages were that the gauze snagged easily and deteriorated in the weather so rapidly that it was good for only one season. A series of square cages, 3' x 3' x 6', using 1 run of the 36" wide gauze on each side wall were used for the tests in 1947 and 1948. A plyboard panel with opening for introducing the weevils and a door to permit access to the interior of the cage was installed on the north side of each cage. (Photo 18).

B. Air Temperatures Inside the Cages. When the first cages were set up there was some concern that the interiors would become so hot during warm spells of weather (maximum temperatures approached 100° F.) that the adult weevils would either die or behave abnormally. There seemed to be considerable hazard of this happening in the large cage (A) with muslin side walls. A thermograph was installed which recorded temperatures both inside and outside of Cage A. Comparisons showed that during the middle of the day temperatures within the cage were from 10 to 15 degrees above those of the outside air. On days when the maximum air temperature was 90°, temperatures within the cage would be from 100 to 105°. These are critical temperatures for the adults of some species of barkbeetles. As a precaution in the weevil tests a lath shade was constructed to cover the top and the east and west side walls of Cage A. During the period of attack temperatures within the cage were watched closely on hot days and when the air temperature approached 100° F., the cage was sprayed with water; evaporation rapidly lowered inside temperatures from 10 to 20 degrees. The effects of this method of cooling lasted for about an hour.

The cages with full screen walls and those made of surgical gauze were well ventilated and did not develop interior temperatures higher than 6 to 8 degrees above the outside air, so no precautions were taken to cool these cages. Observations during the 3 seasons included in these tests have generally indicated that adult weevils can withstand temperatures above 100° F., without injurious effects. The higher the temperature the more active the weevils became and there was no evidence of the adults dying or becoming paralyzed from heat in the cages. Actual tests to determine high temperatures critical to this species of weevil have not been made.

C. Forced Attacks on Internodes Only in Cylinder Cages. During the 1947 season tests were made with small cylinder cages which enclosed the bark area of the internodes only, leaving the nodes and lateral branches exposed. (Photo 19). The purposes for trying out this type of cage were:

a. To develop a small light cage which would require only a small amount of screen, which could be easily installed, and which would permit study of the attack on limited portions of the stem.

b. To see if the weevil could successfully attack when confined to a limited portion of the main stem of the tree. (Access of adults to the needles of the tree under attack was excluded).

c. To see whether or not vigor was an important factor on resistance to weevil attack (trees of highly contrasting vigor were selected for these cages).

The results obtained with this type of cylinder cage are presented in Table VII. In the 2 vigorous ponderosa pine which died (1947 - 1 and 1947 - 2) no successful larval mines could be found in the caged bark areas. However, weevils outside the cages attacked the 1945 nodes and the 1946 internodes which were excluded from the cages. These attacks above the cages developed successful larval mines which entered the wood and may have caused enough injury to kill the trees. For some reason the vigorous trees in this test did not produce much flow of pitch from punctures on the main stem. This was in contrast to the low vigor suppressed trees which survived and produced copious pitch flow on the stem areas enclosed in the cages. No attacks were found outside the cages on any trees except the two ponderosa trees which died.

Results of these tests on caged internodes are inconclusive, but they indicate that:

1. Small cages enclosing separate internodes are difficult to fit to the trees and apparently do not afford suitable conditions for the attack of the weevils.

2. Feeding by weevils on the needles prior to attack on the main stem is necessary in order to condition the tree for successful attack. (In these cages needles plucked from other trees were kept in the cages in order for the adult weevils to complete this phase of their feeding habit).

3. Attack upon the nodes as well as the internodes is important to overcoming tree resistance.

4. Vigor is not the most important factor in tree resistance.

The leads indicated by points 2, 3, and 4 are significant and could be profitably followed up in future studies.

III. INJURY IN THE BARK AREAS AND WOOD RESULTING FROM WEEVIL ATTACK

The 1946 tests demonstrated that some trees which are heavily attacked by the weevil die during the season, while other trees, which appear to be just as heavily attacked, survive and continue growth the following year. A superficial examination of the bark areas where the attack occurs yields little evidence to account for death in some trees and survival in others. It is apparent, however, that something goes on in that part of the tree between the outer bark and the sapwood which produces conditions either favorable or unfavorable to larval growth and development of the weevil. In a search for clues to the conditions that are associated with both susceptibility and resistance, microscopic examinations were made of a series of trees attacked during the 1947 season.

A group of 24 ponderosa pines was enclosed in a single cage into which a weevil population of 1,096 adults was liberated. As soon as the attack was well underway, one tree was cut, removed from the cage, examined carefully under a microscope, and sections of the main stem prepared for micro-slides and further study. This procedure was repeated each week until the latter part of September.

Table VIII summarizes the data obtained by this method of examination and study. It was obvious that part of the trees examined were resisting the attack, others were susceptible, but during the earlier part of the summer it was impossible to distinguish between trees in these two categories. After the last of August, however, it was fairly easy to recognize those trees that were being killed and those that were resisting the attack. In the dying trees larval mines had penetrated to the wood and the larvae were growing rapidly: in the resistant trees the larvae did not get beyond the 1/4 grown stage, larval mines in the green cortex were infiltrated with resins, and the larvae that entered the living cortex were mostly dead or dying.

In Table VIII Tree No. 1 - 10 (Sept. 5) was definitely dying: Tree No. 1 - 13 (Sept. 24) had already been killed by the attack: Tree No. 1 - 9 (August 27) and 1 - 12 (Sept. 19) were resistant: while Tree No. 1 - 11 (Sept. 19) was still doubtful although some of the larvae were dead in resin-filled mines.

In addition to the ponderosa pines listed in Table VIII, similar development was found in a series of 8 Jeffrey pines and 4 Coulter pines that were followed during the 1947 season. In the Jeffrey pines progress of the injury that developed was identical to that in the ponderosa pines.

In the Coulter pines, all of which were resistant, a different type of injury was encountered; the necrotic areas were not localized but all of the outer cortex died soon after the attack. This dead tissue did not penetrate very far, however, and was walled off by a strong layer of cortex, phloem and cambium over the sapwood. No larval mines penetrated into this living tissue.

Sectioning and Staining Techniques used in Study of Injury by Cylindrocopturus in the Bark and Wood.

The sectioning and staining methods used in microscopic studies of the stems were worked out by trial and error until the sections obtained gave good differentiation of the plant tissues with which the study was concerned. The procedures were described in a memorandum prepared in October 1947, which is included here as a matter of record.

"The following staining procedure was adopted in insect resistance studies during the season of 1947 at the Institute of Forest Genetics. When carefully used it differentiates sharply between living tissues of the stem, the xylem, and the necrotic resinous areas resulting from injury caused by adults and larvae of the Cylindrocopturus. The general color pattern in the fully stained and cleared sections gives the following contrasts:

1. Living cells of the cortex, phloem and cambium — bluish green.
2. Xylem — red or purplish red.
3. Necrotic areas — dark brown or purplish brown.
4. Cork and cork cambium — no color.

This staining process was used with young pines up to 1 inch base diameter. In order to retain the form of the larval mines it was necessary to cut sections fairly thick which usually developed considerable warping in the final stages of mounting. This trouble was overcome by clearing from chloroform to balsam and by flattening the sections between two clamped slides before final mounting under a cover glass.

The general procedure was to collect the infested stems as desired, cut them into suitable lengths, store them for a week or two in a solution of alcohol and glycerine, section (without imbedding) on a sliding microtome, and then run the sections through the dehydrating and staining formulae. Following are the steps in the process:

1. Fresh material into (70% alcohol, 3 parts, glycerine, 1 part); leave for 4 or 5 days or longer.
2. Section at about 80 microns — if sections are not stained immediately, store in 50% alcohol to prevent discoloration.

3. Stain for 30 minutes in water solution of safranine and gentian violet — mix by color; should be concentrated to point where solution is not opaque.

4. 50% alcohol — 5 minutes.

5. 70% " " "

6. 85% " " "

7. 95% " " "

8. 100% " " "

9. Stain for 30 to 60 seconds in clove oil, 3 parts, 100% alcohol, 1 part, saturated with fast green.

10. Clear in clove oil 2 parts, 100% alcohol, 1 part, xylene, 1 part. 5 minutes.

11. Clear in 100% alcohol 1 part, xylene, 1 part — 5 minutes.

12. 100% alcohol — 3 minutes.

13. 95% " " "

14. 85% " " "

15. 70% " " "

16. Bleach in 50% alcohol and acetic acid. Acetic acid should be dropped into solution after sections have been immersed; bleach until desired color tone develops, usually requires 3 to 10 minutes.

17. 70% alcohol — 3 minutes.

18. 85% " " "

19. 95% " " "

20. 100% " " "

21. Into 100% alcohol ½ part, chloroform 1 part — 5 minutes.

22. Into chloroform — 5 minutes.

23. Flatten sections if necessary by mounting between 2 slides in xylene and balsam, clamped with clothes pins (3 to 12 hrs.).

24. Mount permanently in balsam under cover glass."

Table I.- Cage tests to determine resistance of pine species to weevil attack.

Designation of Experiment	Species tested	Number of trees	Age (Growing season)	Number of weevils liberated in cage	Number of trees killed by weevil	Survival (First year)
Exp. 1 1947	Pinus ponderosa	11	5th	1,287 adults June 11 to July 7, 1947	4	7 trees (64%)
Exp. M 1948	Pinus ponderosa	19	6th	816 adults June 23 - July 14, 1948	6	13 trees (67%)
Exp. G 1947	Pinus jeffreyi	3	4th	728 adults May 30 - July 11, 1947	1 (killed down to base)	2 trees (66%)
Exp. L 1947	Pinus jeffreyi	14	5th	504 adults July 3 - July 11, 1947	0	14 trees (100%)
Exp. P 1948	Pinus jeffreyi	14	6th	878 adults July 5 - 18, 1948	14	0 trees
Exp. F 1947	Pinus coulteri	10	3rd	498 adults May 30 - July 4, 1947	0	10 trees (100%)
Exp. T 1948	Pinus coulteri	6	4th	1,369 adults July 5 - 10, 1948	0	6 trees (100%)
Exp. K 1947	Pinus lambertiana	1	6th	510 adults June 27 - July 7, 1947	0	1 tree
Exp. J 1947	Pinus torreyana Grafted on P. ponderosa	1	6th	486 adults June 27 - July 7, 1947	0	1 tree

Notes to Table 1.

Exp. 1. P. ponderosa. This cage included 21 trees when installed, but 10 trees were cut and removed during period of attack for examination and sectioning. Mortality could be figured only for the 11 trees left in cage because all of the 10 trees removed were cut before final effect of attack could be determined.

Exp. G. Jeffrey pine. The three trees enclosed in this cage were selected because of their high vigor to see if this was a factor in resistance. All 3 trees were severely injured by the attack which averaged 242 weevils per tree. On the tree listed as dead, the 1947, 1946, and 1945 internodes were killed, but the 1944 internode next to the ground remained alive during the 1948 season.

Exp. L. P. jeffreyi. Twenty trees were originally included in cage; 6 were removed during period of attack. All of the 14 trees left in cage were moderately attacked but resistant.

Exp. P. Pinus jeffreyi. The same cage and the same 14 trees used in Exp. L were used in the 1948 test. These trees were 100% resistant in 1947, but 100% susceptible in 1948.

Exp. P. P. coulteri. On one of these trees the 1947 leader was killed by concentration of adult feeding punctures at its base on the 1946 node. No larvae developed, however.

Exp. M. Pinus lambertiana. This was a vigorous tree in the outplanting east of the Institute. It was heavily attacked and a few larvae developed but did not extend mines. The tree resisted successfully up to the end of the 1947 season; it was cut for examination in November.

Exp. J. Pinus torreyana. A torreyana scion had been grafted to a P. ponderosa stock which extended for one internode above the ground. Attacks were made on both ponderosa and Torrey internodes. Tree resisted but the subsequent 1948 growth was retarded because of injury to main stem.

Table II.- Cage tests comparing resistance of hybrids with parent species.

Designation of Experiment	Species and hybrids tested	Number of trees	Age (growing season)	Number of weevils liberated in cage	Trees killed by weevil attack, 1946	Survival (first year after attack)
Exp. A 1946	Pinus jeffreyi	8	6th	498 adults June 15 - 28, 1946	5	3 trees (37½%)
	Pinus jeffreyi x (P. jeffreyi x P. coulteri	8	6th		0	8 trees (100%)
Exp. E 1947	Pinus jeffreyi	4	7th	523 adults June 9 - July, 1947	1	3 trees (75%)
	Pinus jeffreyi x (P. jeffreyi x P. coulteri)	5	7th		0	5 trees (100%)
Exp. R 1948	Pinus jeffreyi	3	4th	1,505 adults June 28 - July 13 1948	3	0
	Pinus coulteri	9	4th		0	9 trees (100%)
Exp. O 1948	Pinus ponderosa	6	5th	1,190 adults June 23 - July 16 1948	4	2 trees (33%)
	Pinus ponderosa x P. P. scopulorum	12	5th		8	4 trees (33%)
Exp. C 1946	Pinus ponderosa	7	3rd	90 adults June 29 - July 13 1946	0	7 trees (100%)
	Pinus ponderosa x P. P. scopulorum	7	3rd		2	5 trees (71%)
Exp. C-1 1947	Pinus ponderosa	7	4th	585 adults May 25 - July 11 1947	0	7 trees (100%)
	Pinus ponderosa x P. P. scopulorum	5	4th		0	5 trees (100%)
Exp. D 1947	Pinus ponderosa	8	3rd	350 adults May 25 - July 4 1947	0	8 trees (100%)
	Pinus ponderosa x P. apacheca	8	3rd		0	8 trees (100%)
Exp. E 1947	Pinus ponderosa	12	3rd	294 adults May 29 - July 4 1947	0	12 trees (100%)
	Pinus ponderosa x P. apacheca	12	3rd		6	6 trees (50%)
Exp. Q 1948	Pinus ponderosa	10	4th	586 adults June 27 - July 10 1948	3	7 trees (70%)
	Pinus ponderosa x P. apacheca	8	4th		5	3 trees (37%)
Exp. B 1946	Pinus washoensis	8	3rd	281 adults June 20 - July 17 1946	8	0
	Pinus washoensis x P. ponderosa	8	3rd		6	2 trees (25%)
Exp. N 1948	Pinus washoensis	6	5th	542 adults June 25 - July 12 1948	0	6 trees (100%)
	Pinus washoensis x Pinus ponderosa	6	5th		0	6 trees (100%)
Exp. S 1948	Pinus ponderosa	5	4th	546 adults June 29 - July 10 1948	4	1 tree (20%)
	Pinus ponderosa xP. arizonica	2	4th		2	0

Notes on Table 2.

Exp. B. P. jeffreyi vs. P. jeffreyi x (P. jeffreyi x P. coulteri). The Jeffrey pines included in this test were heavily oppressed and shaded in the bottom of the cage. They were apparently only light attacked. The weevil apparently avoids heavily shaded trees and it is doubtful if this test gives a fair comparison between Jeffrey pines and hybrids in the cage.

Exp. B. P. jeffreyi vs. P. coulteri. Two Coulter pine, one vigorous and one suppressed, were cut for examination in October. It was obvious, however, that these two trees had successfully resisted the weevil attack and they are, therefore, included in the mortality count, although only 7 trees remained at the end of the season.

Exp. C-1. P. ponderosa vs. P. macrocarpa x P. P. scopulorum. This cage was set over the surviving trees of Exp. C., conducted the previous season, 1946. The same cage and techniques were used, but about six times as many weevils were introduced to see if the trees would be more susceptible to a heavier attack. Although a good population was established in the cage, some of the weevils appeared to be weak and did not make a normal attack.

Table III.- Unpared species and hybrids of pine attacked in the nursery and arboretum.

Species	Location	Year of attack	Growing season	No. of trees killed by weevil	Success of larval broods
Pinus ponderosa var. scopulorum	Progeny Block and arboretum	1946	14th	21 Mainly internodes since 1942	Good
Pinus ponderosa var. Eldorado x P. ponderosa var. Santa Cruz	Beds 3 and 4 1943 nursery	1948	6th	8	Good
Pinus jeffreyi	Bed 5 1943 nursery	1948	6th	29 (out of 700 trees in bed)	Good
Pinus taeda	Bed 6 1945 nursery	1948	4th	14	Very poor. Few larvae reached the wood layer
Pinus taeda	Bed 7 1945 nursery	1948	4th	3	Poor; a few larvae entered wood near ground level
Pinus echinata x P. taeda	Bed 6 1945 nursery	1948	4th	4	Fair; some larvae entered wood
Pinus rigida x P. taeda	Bed 7 1945 nursery	1948	4th	4	Poor; a few larval mines near ground level
Pinus sylvestris	Small plot in 1944 nursery	1948	5th	12	Good
Pinus monticola x P. strobus	Small plot in 1943 nursery	1948	6th	8	Poor; larvae died out in upper internodes; a few survived near ground level
Pinus montana	Arboretum	1946	15th	3	Fair to good. New adults smaller than in P. ponderosa

Table IV.- Selection shown by weevil in uncaged nursery beds (Tree species paired, planted either in parallel rows or randomized).

Nursery bed number	Species and hybrids Paired	Year of attack	Growing season	Number of trees exposed to attack	No. of trees killed by weevil	Larval development
1941	Pinus murrayana		7th	145	67	Fair
Test	vs.	1946				
Nursery	Pinus murrayana x P. banksiana		7th	16	3	Good
Bed 2	Pinus ponderosa vs.		5th	247	4	Good
1944	P. ponderosa x	1948				
Nursery	P. P. scopulorum		5th	247	17	Good
Bed 3	Pinus ponderosa vs.		5th	220	2	Good
1944	Pinus ponderosa x	1948				
Nursery	P. P. scopulorum		5th	220	3	Good
Bed 4	Pinus ponderosa vs.		5th	245	8	Good
1944	P. ponderosa x	1948				
Nursery	P. P. scopulorum		5th	245	19	Good
Bed 5	Pinus P. scopulorum		5th	220	10	Good
1944	vs.	1948				
Nursery	Pinus ponderosa x P. P. scopulorum		5th	220	5	Good
Bed 8	Pinus ponderosa vs.		4th		6	Good
1945	Pinus ponderosa x	1948				
Nursery	Pinus apacheca		4th		12	Good
Bed 1	Pinus banksiana vs.		4th		20	Good
1945	Pinus murrayana x	1948				
Nursery	Pinus banksiana		4th		4	Fair
	Pinus nigra vs.		4th		3	Poor
Bed 9	Pinus murrayana x	1948				
1945	(P. banksiana x					
Nursery	Mind)		4th		12	Good

Table V.- Development of Cylindrocopturus larvae in pines resistant to attack season of 1946.

Tree number and species	Period of attack	Date of first examination and condition of larvae	Development after tree was cut
x-1. Pinus jeffreyi x (P.J. x P. coulteri	July August 1946	October 24, 1946 First stage larvae in necrotic areas	December 26, 1946 Mostly full grown larvae in wood
x-2. Pinus jeffreyi x (P. J. x P. coulteri)	"	November 28, 1946 First stage larvae in necrotic areas	Dec. 13, 1946 — Larvae extending mines in green phloem. Dec. 26, 1946 — Some mines have reached cambium. Feb. 1, 1947 - Larvae full grown
x-3. Pinus jeffreyi x (P. J. x P. coulteri)	"	November 30, 1946 First stage larvae in necrotic areas	Dec. 26, 1946 — A few mines entering green cortex. Feb. 10, 1946 — Larvae 1/4 to 3/4 grown; a few mines entering wood.
x-8. Pinus jeffreyi x (P. J. x P. coulteri)	"	March 5, 1947 First stage larvae in necrotic areas	Mar. 14, 1947 — A few small entering cortex. Apr. 7, 1947 — All larvae dead, none completed mines.
x-9. Pinus jeffreyi x (P. J. x P. coulteri) (4 trees)	"	March 20, 1947 First stage larvae, many dead	Apr. 14, 1947 — All larvae dead.
x-10. Pinus jeffreyi (4 trees)	"	March 20, 1947 First stage larvae many dead	Apr. 14, 1947 All larvae dead
x-7. Pinus murrayana	"	March 5, 1947 First stage larvae in necrotic areas	Apr. 7, 1947 — Larvae mostly dead; a few completed mines and entered wood

Table VI.- Development of Cylindrocopturus larvae in trees resistant to attack season of 1948.

(Trees of different species cut October 2, 1948, to determine whether first stage larvae would start growth)

Tree No. and Species	Period of attack	Date trees were cut	Development after trees were cut
48/26 Pinus jeffreyi	July August 1948	October 2 1948	Nov. 17, 1948 — Larvae extending mines in green cortex and phloem. Dec. 16, 1948 — Full grown larvae in wood, heavy brood
48/27 Pinus jeffreyi	"	"	Nov. 17, 1948 — No larvae were found, attack light. Dec. 16, 1948 — A few full grown larvae were found which had entered wood.
K-7 Pinus ponderosa	"	"	Nov. 17, 1948 — Larval mines in green phloem and cambium. Dec. 16, 1948 — 3/4 to full grown larvae extending mines on surface of sapwood and entering wood.
O-17 Pinus ponderosa x P. P. scopulorum	"	"	Nov. 17, 1948 — 3/4 grown larvae, mines on sap wood surface and entering wood. Dec. 16, 1948 — Full grown larvae in wood: heavy brood.
K-9 Pinus coulteri	"	"	Nov. 17, 1948 — No larval development found. Dec. 16, 1948 — A few mines found which reached sapwood surface but all larvae died.
K-11 Pinus coulteri	"	"	Nov. 17, 1948 — Some larval developmer found in this small suppressed tree Dec. 16, 1948 — 10 successful mines found on 1947 internode; larvae have entered wood.

Table VII.- Effect of weevil attack when limited to internodes of pines of different vigor.

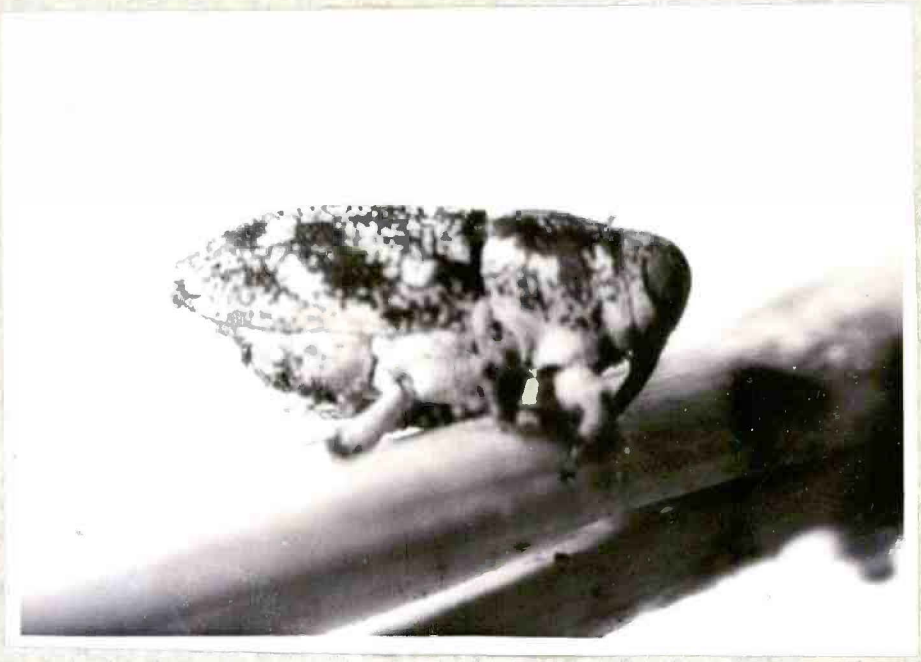
Tree No.	Species	Vigor	Effects of attack
1947-1	P. ponderosa	High vigor	Tree died; no successful larval mines in caged area.
1947-2	P. ponderosa	High vigor	Tree died; no successful larval mines in caged bark area.
1947-3	P. ponderosa	Medium vigor	Tree survived; no larval development.
1947-5	P. ponderosa	Suppressed; low vigor	Tree survived; no larval development.
1947-6	P. ponderosa	Suppressed; low vigor	Tree survived; no larval development.
1947-9	P. ponderosa	Suppressed; low vigor	Tree survived; no larval development.
1947-11	P. jeffreyi x (PJ x PC)	High vigor	Tree survived; no larval development.
1947-12	P. jeffreyi x (PJ x PC)	Medium vigor	Tree survived; no larval development.
1947-13	P. jeffreyi x (PJ x PC)	Suppressed low vigor	Tree survived; no larval development.
1947-16	P. coulteri	High vigor	Tree survived; no larval development.

Table VIII.- Development of injury in the main stems of ponderosa pines during progress of weevil attack season of 1947.

Tree No.	Date tree was cut	Stage of weevil development	Injury in main stem of attacked internodes
1 - 1	Jul. 3, '47	Adults on outer bark	Small cavaties in cortex under punctures in epidermal bark layer
1 - 2	Jul. 10, '47	Adults on outer bark	Large necrotic areas around punctures
1 - 3	Jul. 15, '47	Adults on outer bark; a few eggs	Small necrotic areas developing around punctures
1 - 4	Jul. 21, '47	Adults on outer bark	Necrotic areas developing around punctures
1 - 5	Jul. 31, '47	Adults on bark eggs; first stage larvae	Large necrotic areas in cortex, some containing small larval mines
1 - 6	Aug. 6, '47	Adults on bark eggs; large to 1/4 grown	Large necrotic areas; small larvae extending mines in necrotic areas and into green cortex
1 - 7	Aug. 14, '47	Adults on bark Larvae to 1/4 grown	Large necrotic areas; larvae extending mines in necrotic areas and through green cortex
1 - 8	Aug. 20, '47	Adults on bark eggs; larvae to 1/4 grown	Large necrotic areas; larvae extending mines in necrotic areas and through green cortex
1 - 9	Aug. 27, '47	Larvae to 1/4 grown, mostly dead	Large necrotic areas; larval mines in green cortex heavily infiltrated with pitch
1 - 10	Sept. 5, '47	Adults on bark eggs; larvae up to 1/2 grown, numerous	Large necrotic areas; many well developed larval mines in cortex, some of which have penetrated phloem and cambium layers and have scored surface of sapwood
1 - 11	Sept. 11, '47	Adults on bark Larvae to 1/4 grown	Large necrotic areas; larval mines extending into green cortex
1 - 12	Sept. 19, '47	Adults on bark Larvae to 1/4 grown, many are dead	Large necrotic areas; larval mines in green cortex infiltrated with pitch
1 - 13	Sept. 24, '47	Larvae, 1/2 to full grown	Large necrotic areas; phloem and cambium areas mostly dead; larval mines have destroyed much of the inner bark tissues; some mines have entered wood

THE PINE REPRODUCTION WEEVIL

Cylindrocorturus eatoni



1/231 E

Photo 1

Adult in Feeding Position on Pine Needle

This weevil attacks and kills a number of species of western pines but its importance is recognized mainly in ponderosa and Jeffrey pines. It attacks only young trees. Several large plantations of ponderosa and Jeffrey pine in northern California have been almost completely wiped out by this insect.

When the adults emerge in the spring (specimen pictured above is enlarged about 25 times) from dead trees in which they have overwintered as larvae, they go first to the needles of living trees where they make punctures in the epidermal tissues. This is considered to be a feeding habit which lasts for about 10 days. The adults then go to the main stems and branches where they make similar punctures and deposit eggs. Larvae develop rapidly during the summer and are mature by early fall. (See Life History Chart, Photo 5.)

DAMAGE TO NATURAL
PINE REPRODUCTION

In nature the pine reproduction weevil can be found in nearly all ponderosa and Jeffrey pine stands where there are young trees. In un-even age stands it causes only a minor amount of damage. Where there are even-aged stands of young reproduction, weevil populations may become epidemic and kill a high percent of the young trees.

These three views were taken on the Harris Ranch burn of the Sierra National Forest. The fire occurred in 1934, and within a few years a dense stand of ponderosa pine reproduction came in on the burned area. During the period from 1938 - 1945 the weevil destroyed a large part of this natural reproduction.

13197 D

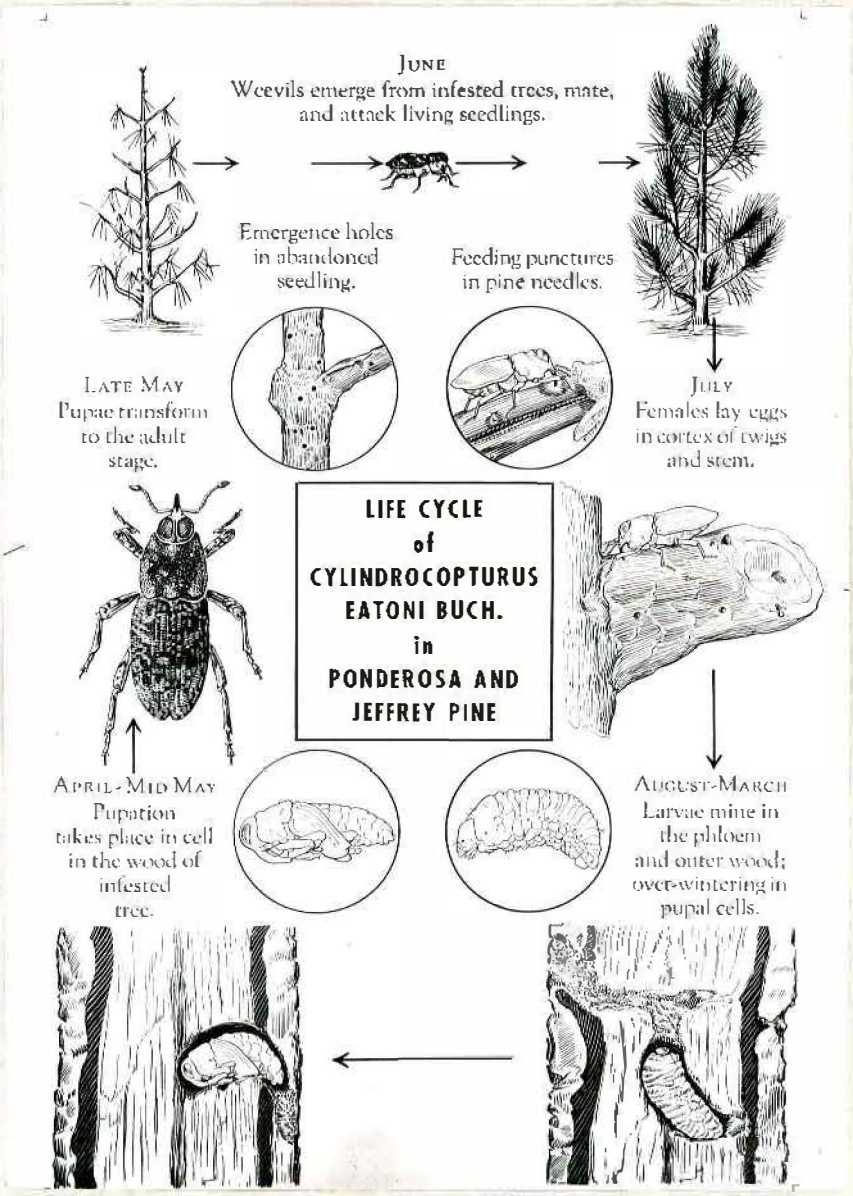
Photo 2

13197 C

Photo 3

13197 B

Photo 4


JUNE
Weevils emerge from infested trees, mate, and attack living seedlings.
Emergence holes in abandoned seedling.
Feeding punctures in pine needles.
LATE MAY
Pupae transform to the adult stage.
JULY
Females lay eggs in cortex of twigs and stem.
LIFE CYCLE
of
CYLINDROCOPTURUS
EATONI BUCH.
in
PONDEROSA AND
JEFFREY PINE
APRIL-MID MAY
Pupation takes place in cell in the wood of infested tree.
AUGUST-MARCH
Larvae mine in the phloem and outer wood; over-wintering in pupal cells.

11525

Photo

Figure 5.

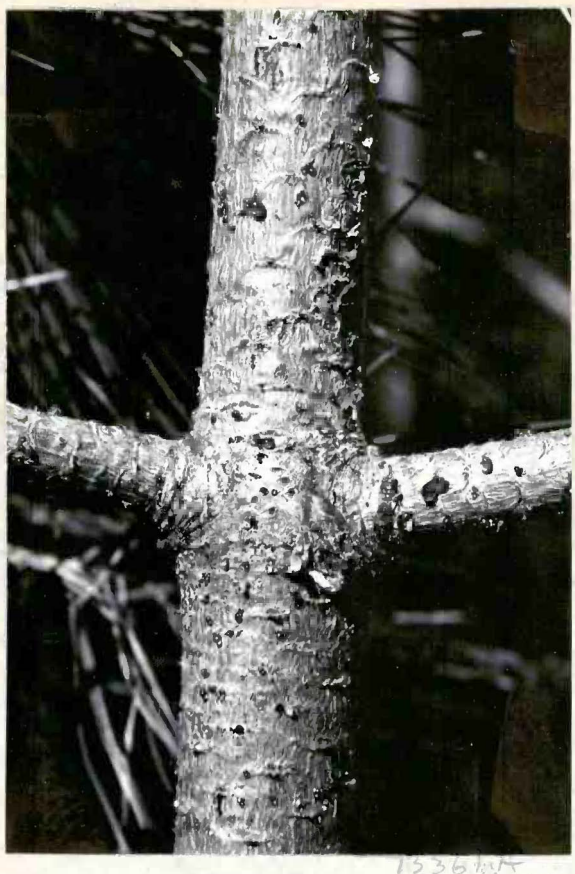


Photo 6.

Photo 7.

RESIN GLOBULES ON THE BARK SURFACE ARE THE MOST CONSPICUOUS EVIDENCE OF WEEVIL ATTACK

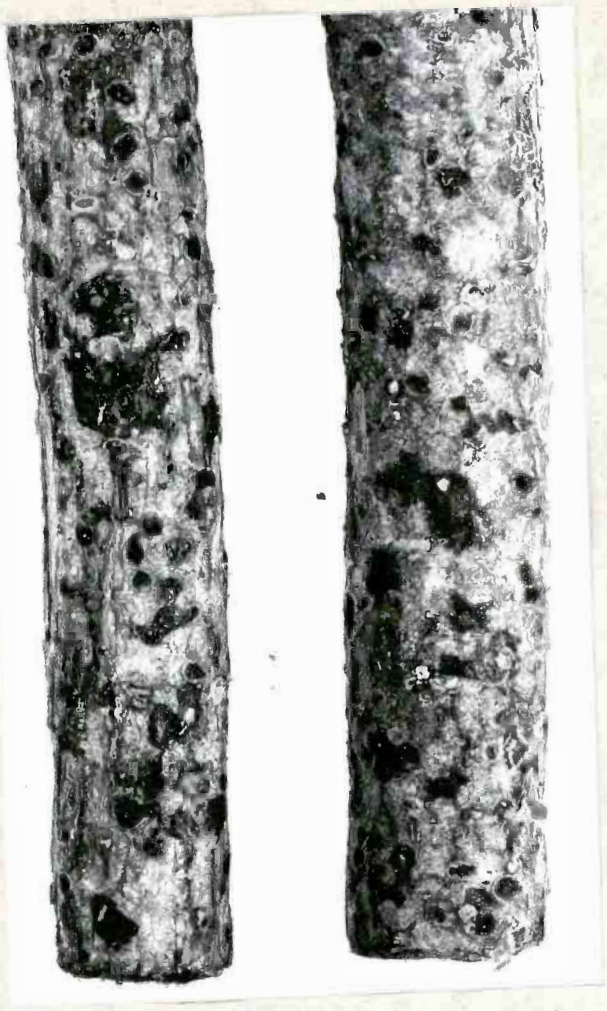
When punctures made by the adult weevil sever a resin duct in the outer cortex, the resins flow out and form a glistening bead of pitch on the outer bark surface. Resistant trees often produce copious pitch flow.

Photo 6. Resin globules formed on the node and internodes of a resistant Jeffrey-Coulter hybrid.

Photo 7. Numerous resin globules on the internode of a resistant Coulter pine.

Photo 8. Heavy pitch flow on a suppressed but resistant ponderosa pine.

Photo 8.



13350-A

Photo 9.

13314-B

Photo 10.

NECROTIC AREAS FORM AROUND PUNCTURES IN OUTER BARK

Soon after the punctures are made by the weevil in the outer bark, the living tissues of the cortex bordering the cavity begin to die. This dying tissue often spreads through a considerable area around the puncture. The affected areas are made up of resin impregnated cells, dark brown in color, which contrast sharply with green living cortex.

Photo 9 shows a ponderosa pine stem with the epidermal layer of bark removed to expose the necrotic areas in the cortex. This picture was taken about 15 days after the attack was started; some of the necrotic are not yet fully developed. When fully formed the areas vary greatly in size, some never becoming larger than small brown spots around the punctures.

Photo 10 is from the stem of a ponderosa pine after the necrotic areas are fully formed, about 4 weeks after attack. The first instar larvae usually feed in these areas instead of in the living cortex.

1 11231 D'
2 13197 D
3 13197 C
4 13197 B
11525 chart
7 – 13361 A
8 – 13361 B
9 – 13361 C
5 – 13272 B
6 – 13351 A
10 – 13350 A
11 – 13350 B
12 – 13314 C
13 – 13393 A
14 – 13341 H
15 – 13341 A
16 – 13341 B
17 – 13341 C
18 – 13341 D
19 – 13343 A
20 – 13347
21 – 13349 C
22 – 13340 A
23 13341 F
24 13341 G

25 – 13344 A
26 – 13342

CF&RES

CALIFORNIA FOREST & RANGE EXPERIMENT STATION

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Designation or Subject

MEMORANDUM FOR .....

Photo 11. 133119C

Photo 12. 133193A

LARVAL MINES AND MATURE LARVAE IN SUSCEPTIBLE PONDEROSA PINE KILLED BY THE PINE REPRODUCTION WEEVIL

After the adult weevils begin their attacks on the stems, eggs are deposited in small cavities in the outer bark tissues, either in or alongside of necrotic areas. After the eggs incubate the tiny larvae feed first in the dead necrotic areas, then extend mines into the living cortex. In susceptible trees the larvae grow rapidly during July and August, extend their mines into the phloem and cambium layers, and by the last of September are full grown. They burrow into the wood to overwinter.

Photo 11 shows scoring of larval mines on sapwood surface (left) and in the inner bark (right). The holes into the wood were made by the full grown larvae when they started their overwintering burrows. This tree was killed when about 14 years of age and the section pictured is from an internode containing 5 annual wood rings.

Photo 12. This picture is about natural size and shows weevil larvae in a section which includes part of the terminal shoot of a young pine. In small stems the overwintering larvae usually burrow through to the pith.

INJURIES CAUSED BY NECROTIC AREAS IN STEMS OF RESISTANT TREES

Photo 13. Main stem of Jeffrey-Coulter hybrid which resisted attack by weevil in June 1947. (Stems were photographed in June 1948). Some of the dried resin globules still adhere to the bark surface. The location of necrotic areas in the cortex is shown by sunken, roughened areas in the outer bark.

Photo 13 13349C

Photo 14. Enlarged cross-section from main stem of Jeffrey-Coulter hybrid which resisted attack of weevil in June 1946. Cross-section was made in June 1948, approximately 2 years after the attack.

One of the old necrotic areas is shown on the bark surface at the top of this section. This area (N) was effectively walled off from the living cortex during the 1946 season, but was partly mined by a first stage weevil larva. During the growing seasons of 1947 and 1948 the necrotic areas was pushed outward by the growing cortex underneath the dead tissues. These old necrotic areas gradually separate from the outer bark and eventually are sloughed off.

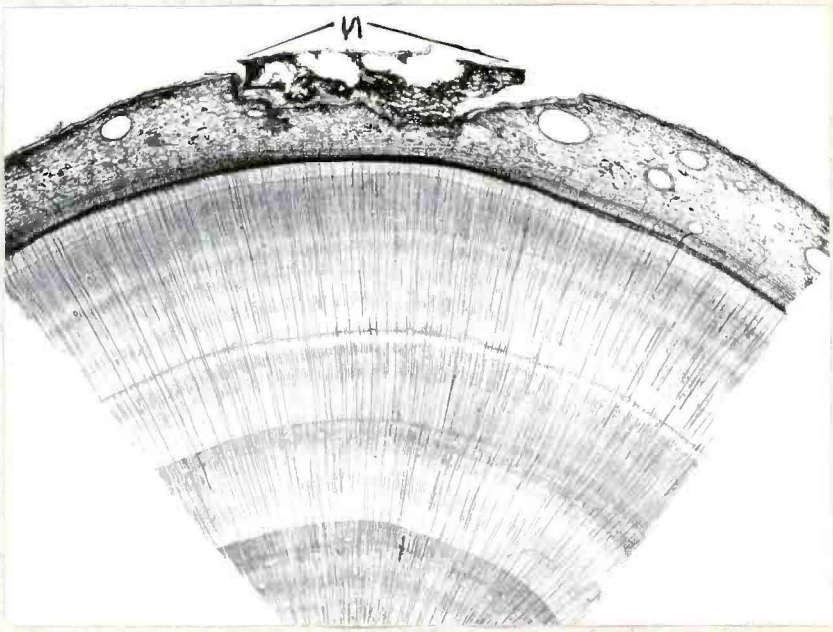


Photo 14 13340-A

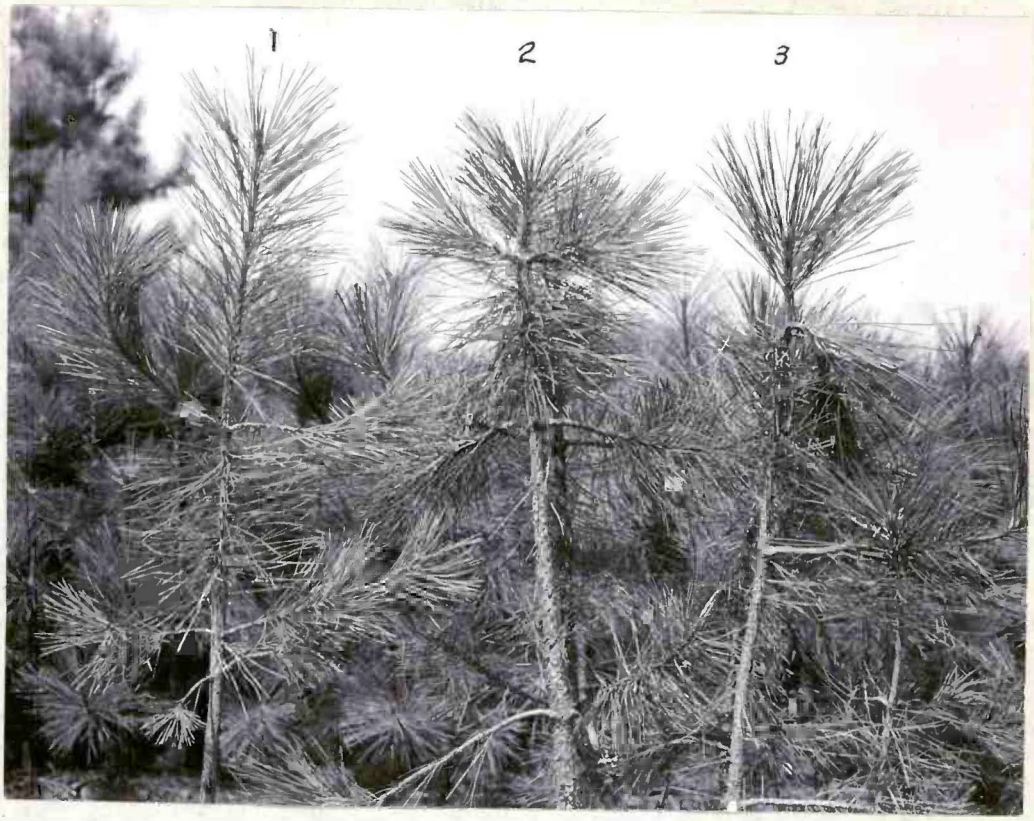


Photo 15. 43347

RESISTANT PONDEROSA PINES, HEAVILY ATTACKED BY THE WEEVIL IN JUNE AND JULY 1947, SHOWING SET-BACK OF TERMINAL GROWTH IN 1948.

Trees 2 and 3, at right, were attacked and numerous necrotic areas formed on the 1945 and 1946 internodes. These trees survived the attack, however, and continued to grow the following season, although the 1948 growth was greatly reduced from that of the preceding year (1947).

Tree 1, at left, of the same age and growing in the same nursery bed, was not attacked in 1947. In contrast this tree exhibits normal leader growth for both seasons of 1947 and 1948.

TYPES OF CAGES USED IN NURSERY AT THE INSTITUTE OF FOREST GENETICS TO TEST PINES FOR RESISTANCE TO WEEVIL ATTACK.

Figure 16. This cage was made up with top and sides covered with 24-mesh screen; the ends were closed with plyboard. Lath were used to shade the top on hot days.

Photo 16 13272B

Figure 17. This cage enclosed Jeffrey-Coulter hybrids which had reached a height over 6 feet during the 1946 growing season. Shortage of 24 mesh screen made it necessary to cover the sidewalls with muslin. This type of cage produced interior temperatures that were almost 20° F. higher than the outside air during the heat of the day. It was feared that these high temperatures would be detrimental to both trees and insects. As a precaution a thermograph, which recorded inside temperatures, was installed, and when a temperature around 100° was reached, the cage was cooled down by spraying with water. It was found, however, that the pine reproduction weevil can endure fairly high temperatures without perceptible injury. No special precautions were taken to control cage temperatures after the 1946 season, as surgical gauze instead of muslin was used in lieu of fine mesh-wire screen.

Photo 17 13273A

TYPES OF CAGES USED IN NURSERY AT INSTITUTE OF FOREST GENETICS TO TEST PINES FOR RESISTANCE TO WEEVIL ATTACK.

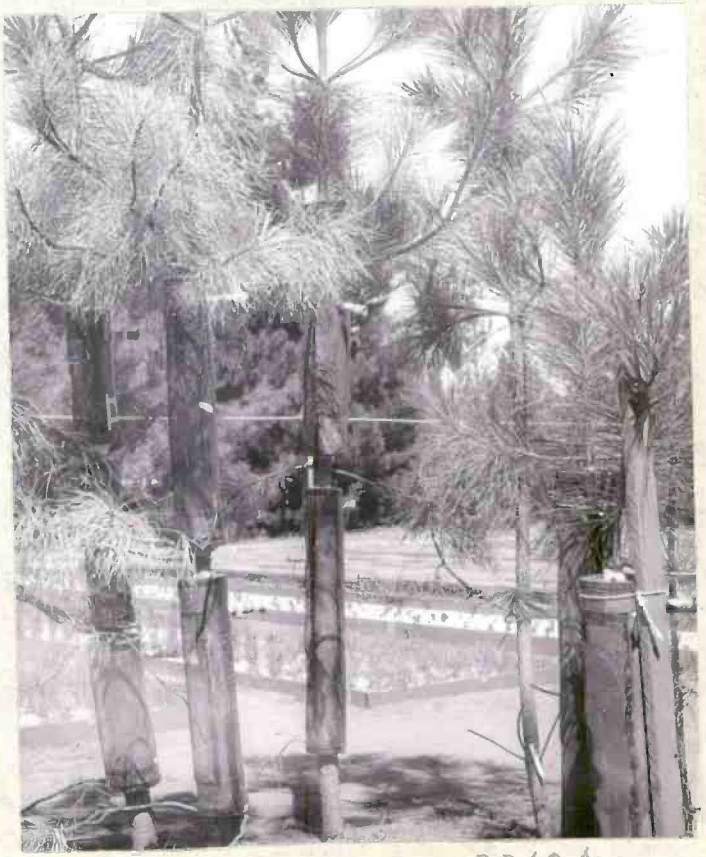
Photo 18. This type of cage was used for most of the tests in 1947 and 1948. The dimensions were 3' x 3' x 6', covered with 24 x 28 mesh surgical gauze. A ply-board panel was fitted to the north side with a bolted door to permit access to the cage interior. A Mason jar cap was fitted into the panel; adult weevils reared from infested trees in an insectary were collected in the jar which was then screwed into the panel to permit the weevils to enter the cage.

This type of cage was fairly satisfactory but the gauze covering was good only for one season.

13351A

Photo 18

Photo 19. Small cylinder cages, made up of 24 mesh screen and cardboard were tried during the 1947 season. These enclosed only the internodes and the branches and nodes were excluded. It was found that the weevils could not or would not establish their attack in these cages. Plucked needles were put in the cages to provide for the feeding requirements of adults, but most of them died within two or three weeks. No trees were killed except those that were attacked by weevils outside of the cages.



13362A

Photo 19

Photo 20. COMPARISON OF VIGOROUS AND SUPPRESSED PONDEROSA PINES EXPOSED TO WEEVIL ATTACK.

The trees shown here are all of the same age. A nursery bed with trees in the 6th growing season was thinned in 1947, leaving only a few of the dominant and suppressed trees. Weevil attack was forced on these trees in cylinder cages enclosing only the internodes (Photo 19). Two of the dominant trees were killed, apparently by weevils from outside of the cages. The small suppressed trees all resisted the attack. This test was not conclusive because of caging technique that was used, but the result supports other indications that vigor alone is not a dominant characteristic of resistance.

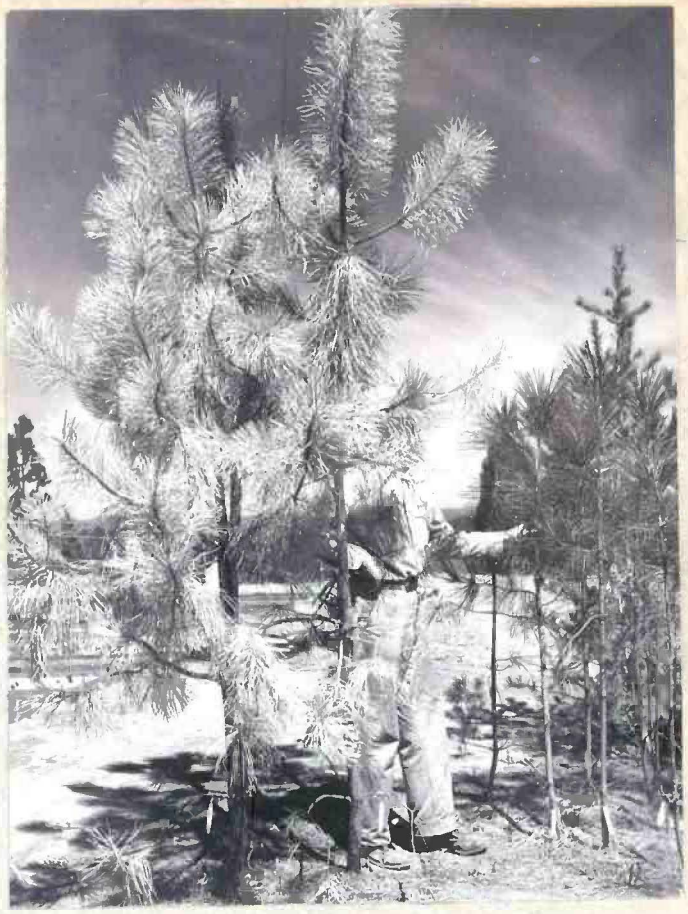


Photo 20

Photo 21. THIS GROUP OF 14 JEFFREY PINES WERE ALL RESISTANT IN 1947, BUT ALL TREES WERE SUSCEPTIBLE IN 1948.

When the cage was removed from this group of trees in Sept. 1948, all trees were dead. During the previous season of 1947 the same trees had been subjected to attack by 504 adult weevils, but all trees resisted the attack.

In 1948 the test was repeated with the same cage and trees, but this year 878 weevils were introduced into the cage and all trees were killed. It is believed that a change in the condition of the trees, rather than the increase in weevil population, accounts for the susceptibility found in 1948.

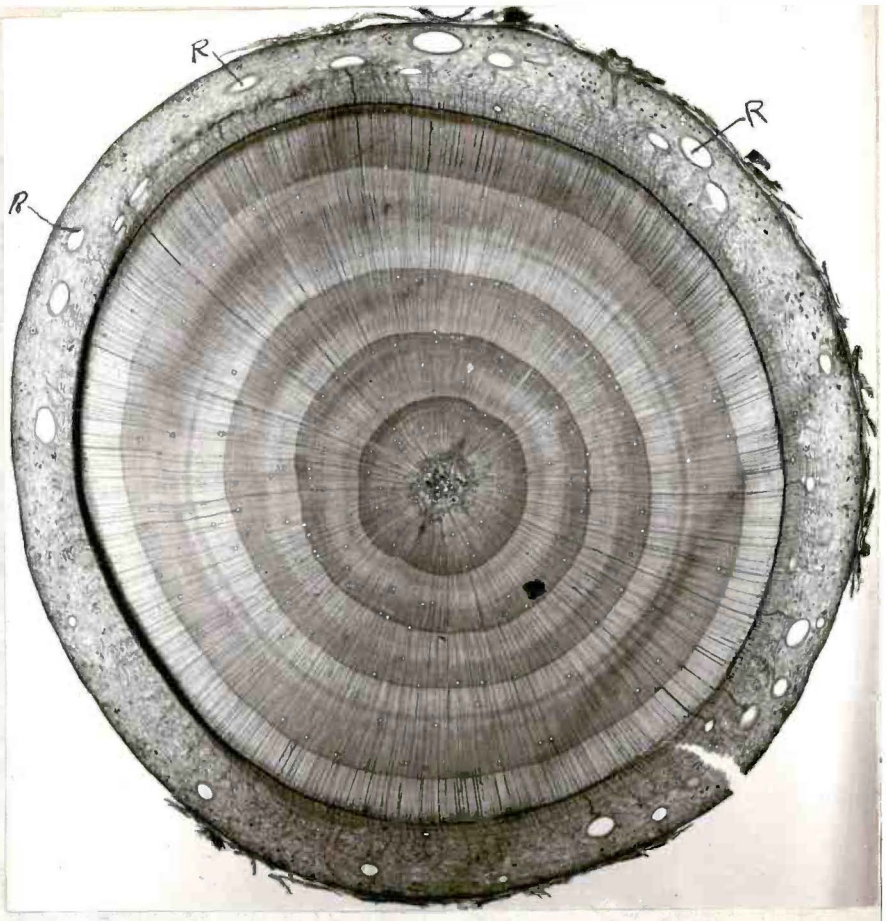
Photo 21

CROSS-SECTIONS FROM MAIN STEMS OF PONDEROSA PINES COMPARING A NORMAL TREE WITH A TREE RECENTLY ATTACKED BY WEEVIL

Photo 22. This cross-section is from the main stem of a normal healthy tree in its 6th year of growth.

~~Resin ducts (R) are the only openings to be seen in the cortex and phloem areas.~~

This tree has not been attacked by the weevil. The openings (R) in the bark are resin storage vessels, sometimes known as "pustules". They are fairly short longitudinally and irregular in form and size.



13341 H

Photo 22

Photo 23. Cross-section from main stem of a tree about 1 week after attack by adult weevils. This section shows not only the resin ~~ducts~~ (R), but also the punctures and cavities (P) made by the mandibles of adult weevils in their feeding activity in the outer cortex.

(vessels)

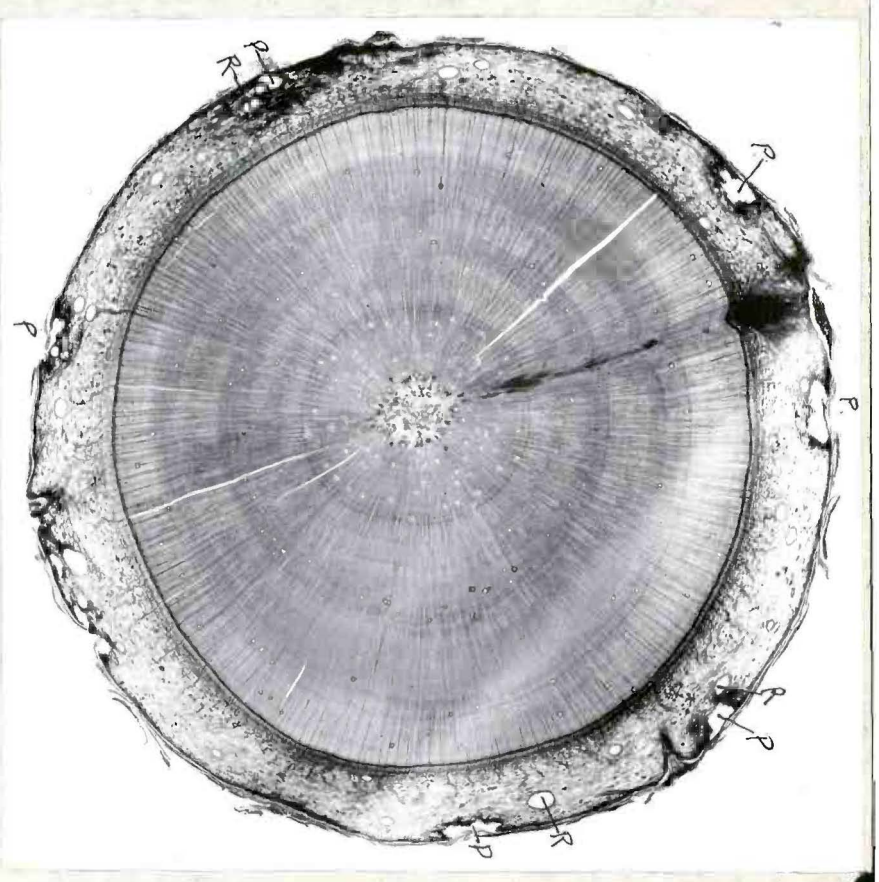


Photo 23. 13341 A

DEVELOPMENT OF NECROTIC TISSUE AROUND PUNCTURES MADE BY THE WEEVIL IN THE OUTER BARK OF PONDEROSA PINES.

Photo 24. The first phases of the development of necrotic areas. This tree was sectioned about 15 days after it was attacked by the weevil. The necrotic areas (N) first appear as discolored tissue around the punctures and cavities (P) made by the weevil in the cortex. In this tree the necrotic areas have not as yet been walled off from the living tissue by formation of a protective layer of cork cells.

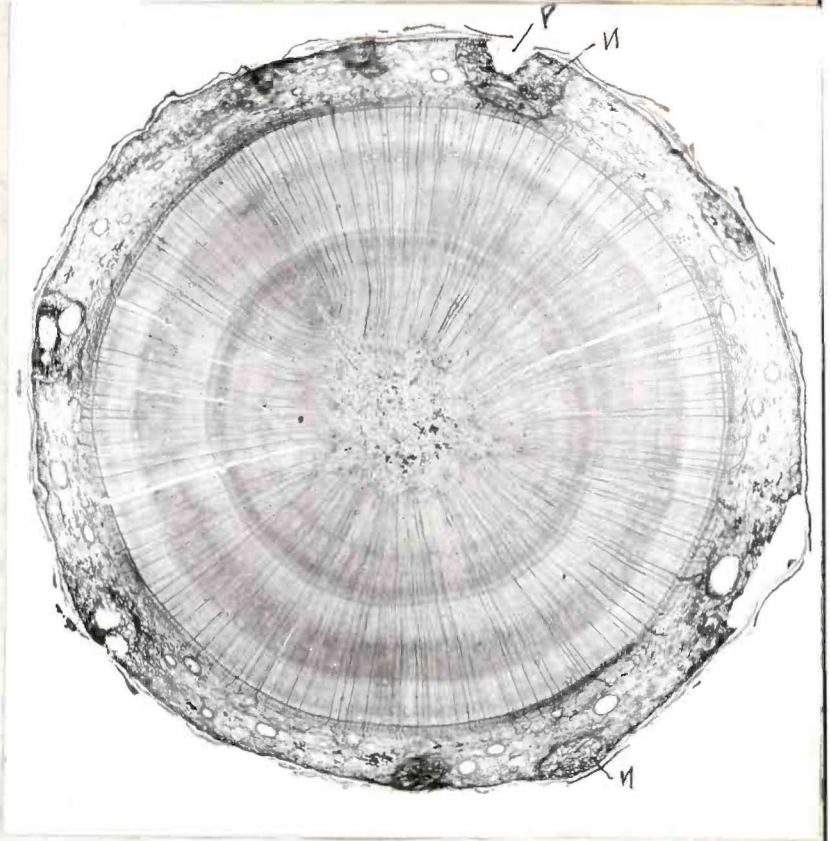


Photo 24 P3341 B

Photo 25. This tree was sectioned about 30 days after the attack was started. Most of the necrotic areas (N) are completely formed, are composed of dead, dark brown, resin-impregnated cells, and have been walled off from the living tissue by a layer of cork cells. The first larval mines (L) have appeared in some of the necrotic areas. This section also shows a concentration of small resin ducts (R) which are forming in the wood just under the cambium.

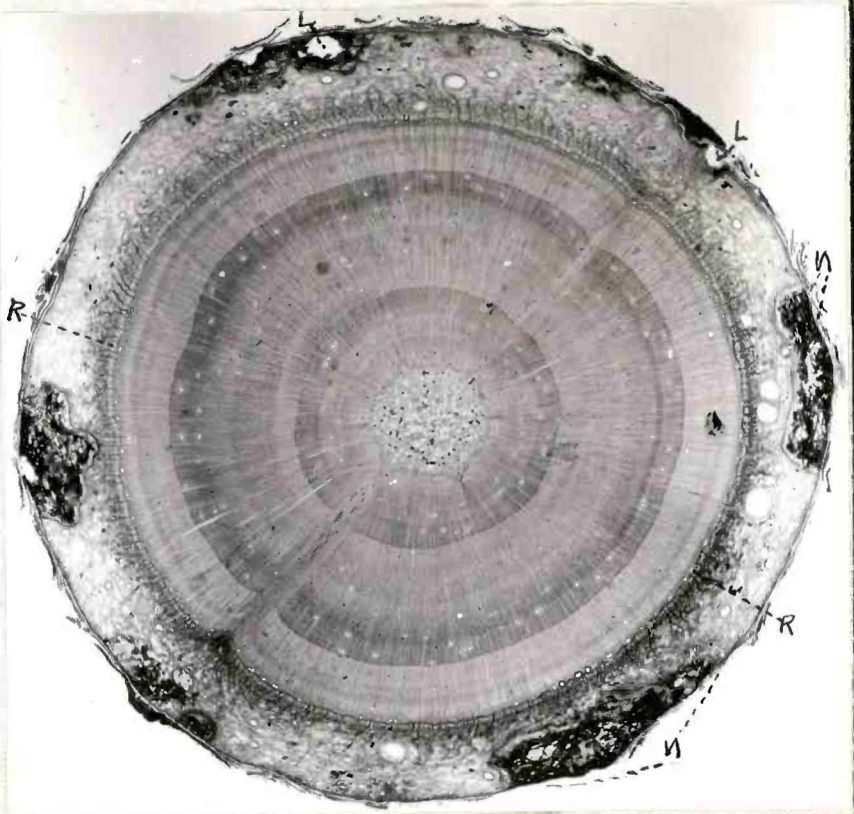


Photo 25 P3341 C

ADVANCED STAGES OF NECROTIC AREAS AND LARVAL MINES IN SUSCEPTIBLE PINES ATTACKED BY WEEVIL.

Photo 26. Cross-section from ponderosa pine about 50 days after tree was attacked by the weevil. Necrotic areas are greatly extended and larval mines are well developed.

L - larval mines in necrotic areas.
L-2 - larval mine which has broken out of necrotic area, entered the cortex and reached sapwood.
C - cork layer separating necrotic area from living cortex.
R - resin ducts in older wood layers.

In this tree there is no concentration of resin ducts in the outer wood layer.

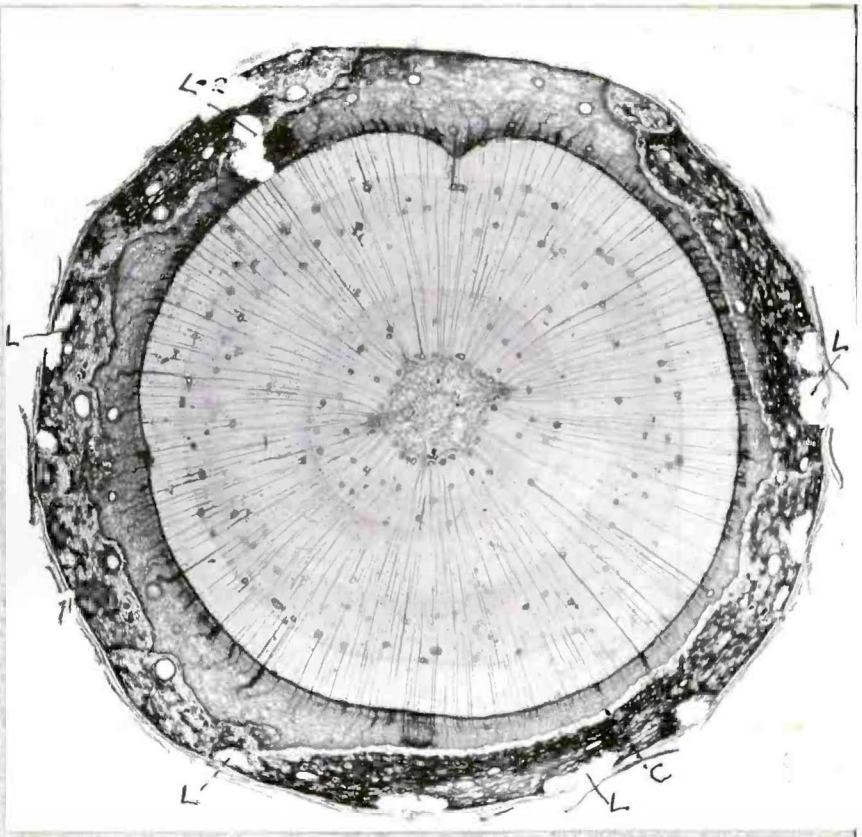


Photo 26 13347 D

Photo 27. Cross-section from stem of Jeffrey pine about 90 days after tree was attacked. All of the cortex, phloem and cambium area is dead. The larval mines (L) are extensive throughout the dead cortex and phloem areas and some have entered the sapwood. This tree formed very small resin ducts (R) in the outer sapwood, indicating that the tree put up some resistance; however, these ducts are considerably smaller than those found in normally resistant trees.

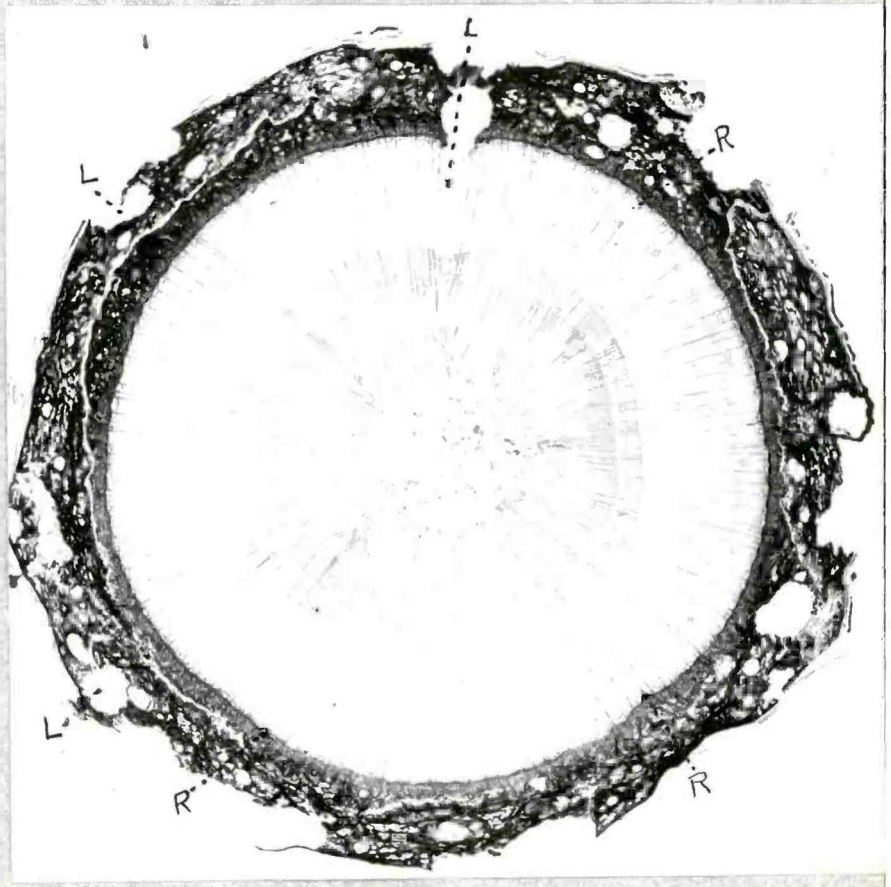


Photo 27 13343 A

RESISTANT PONDEROSA PINES FORM HEAVY CONCENTRATIONS OF RESIN DUCTS IN OUTER WOOD RING.

Photo 28. This tree was attacked in July 1947; section was taken in May 1948. Necrotic areas have been completely walled off and a ring of large resin ducts (R) formed in the outer wood just under the cambium. Continued wood growth following the attack has buried these resin ducts well within the wood layers.

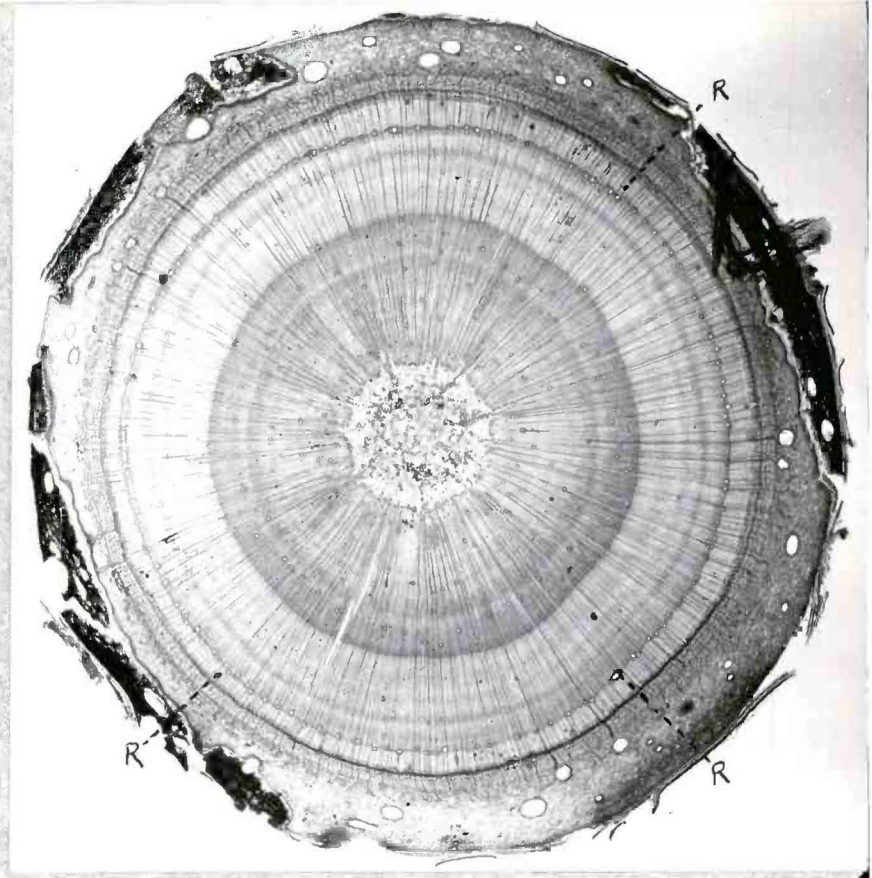


Photo 28 13341F

Photo 29. This tree was attacked by the weevil in May and June 1947: section was taken in September 1947. The ring of large resin ducts has been enclosed by wood growth of the 1947 season. Necrotic areas have been completely walled off and are separating from the living cortex. No successful larval mines appear in these sections.

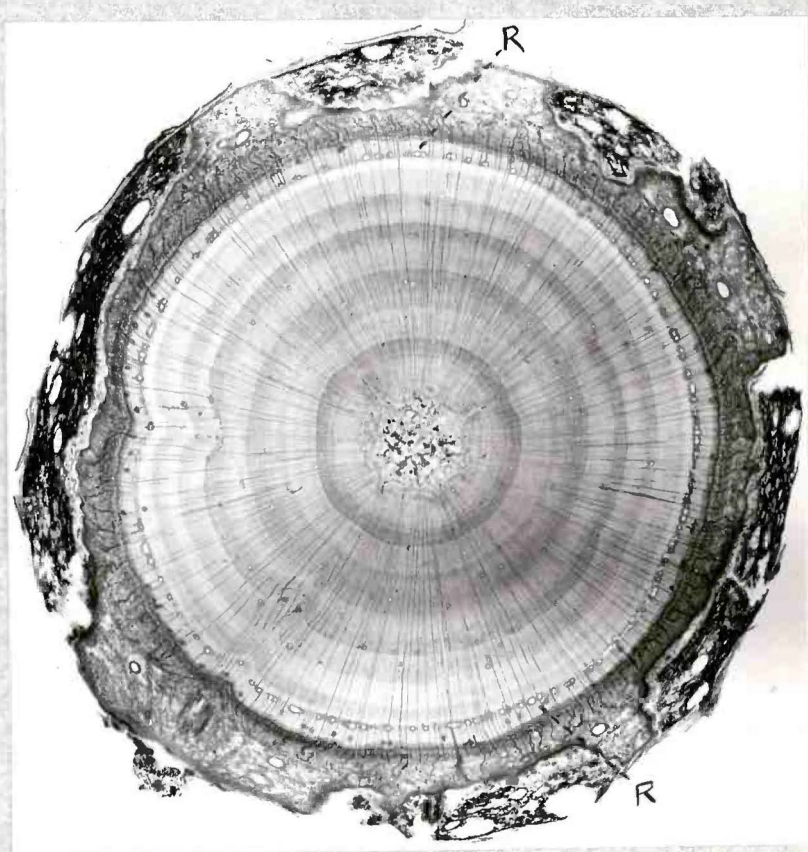


Photo 29 13341 G

COULTER AND SUGAR PINES RESISTANT TO WEEVIL ATTACK.

Photo 30. This Coulter pine was attacked in June 1947: section was taken October 27, 1947.

The necrotic areas differ from those in ponderosa pine. It is characteristic of Coulter pine that the necrotic tissue forms a complete band in the outer cortex with a strong underlying layer of living cortex and phloem. In this section the band of dead necrotic tissue is being pushed out and broken up by the vigorous growth of underlying tissue. One larval mine (L) in the necrotic area has been walled off. A strong ring of resin ducts (R) has become imbedded in the 1947 wood growth.

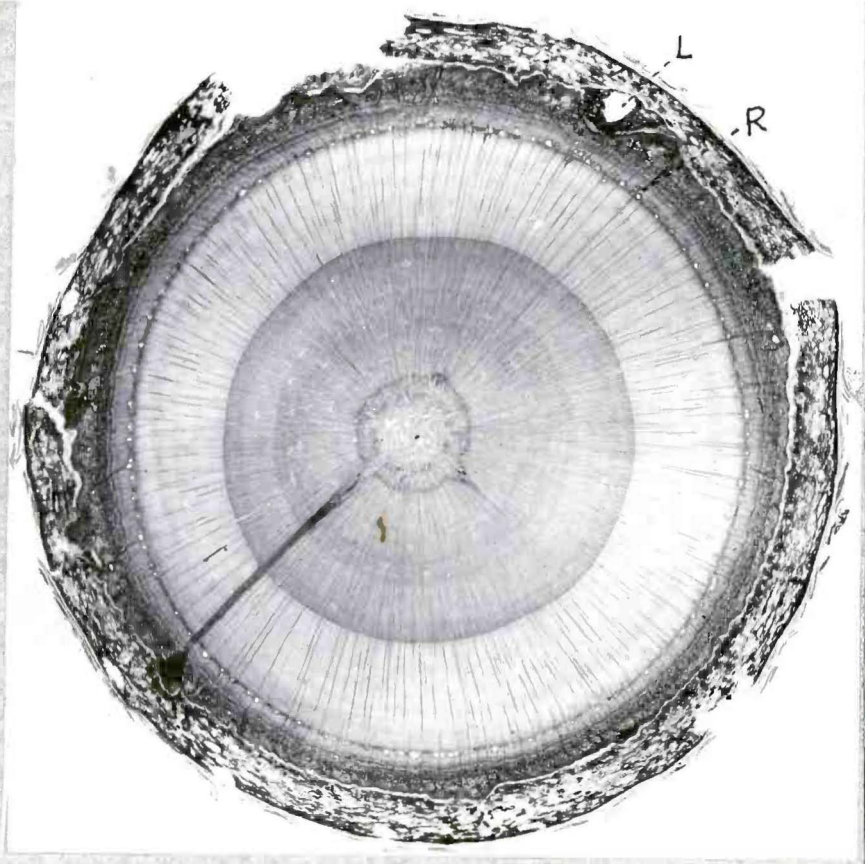


Photo 30 13344A

Photo 31. This sugar pine was attacked in July 1947: section was taken October 22, 1947. The necrotic area in sugar pine is quite similar to that in Coulter pine, but the resin ducts in the wood are less concentrated.

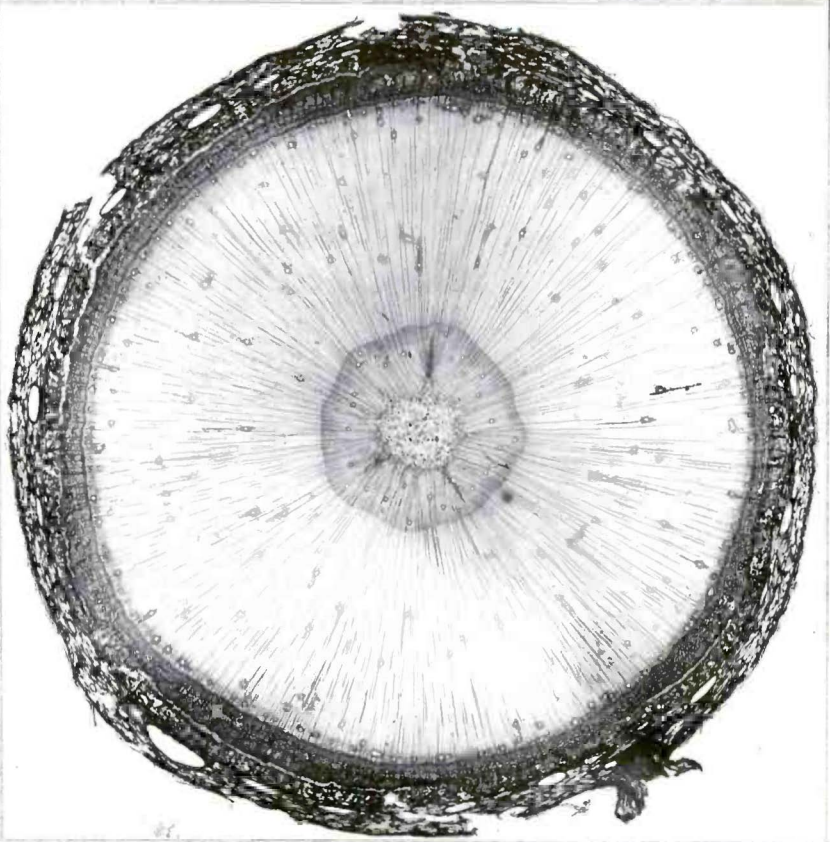


Photo 31 13342